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The Effect of Insulin-Like Growth Factor-1 and Dietary Fat on Disaccharidase Activity and Membrane Fatty Acid Composition of the Suckling Rat Small Intestine

by

© Shelley Anne Kit

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science in Nutrition and Metabolism

Department of Agriculture, Food and Nutritional Science

Edmonton, Alberta

Fall 1997

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Degree:	Master of Science in Nutrition and Metabolism
Year Degree Granted:	1997

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Date: April 28, 1997

ABSTRACT

It has been suggested that Insulin-Like Growth Factor-I (IGF-I) plays an important role in small intestine development. To determine if a physiological dose of IGF-I could influence sucrase and lactase activity levels, suckling rat pups were treated with an oral gavage of IGF-I. Four diets differing in fat composition were fed to lactating dams. Brush border membrane was isolated from jejunal and ileal segments of suckling rat small intestine. Fatty acid analysis of choline, ethanolamine, and serine intestinal phospholipids was performed. IGF-I treatment had no effect on intestinal sucrase and lactase activities. Changes in diet fat indirectly effected the fatty acid composition of suckling pup intestine. IGF-I was observed to effect C20:4(6) levels in ileal segments. A relationship was observed between lactase activity and membrane C20:4(6) and C22:6(3) levels. Many of the hypotheses stated were not confirmed by oral IGF-I or diet fat treatments.

ACKNOWLEDGMENTS

The completion of this thesis would not have been possible without the insight and guidance of my supervisor, Dr. M.T. Clandinin. Special thanks are given to Dr. A.B.R. Thomson whose knowledge and friendship were invaluable to me and to my parents whose love, support, and encouragement have guided me. I would also like to extend my sincere thanks to Dr. Goh, Kelly, Monika, Kim, and Elizabeth for all their help and support and to a few very special friends, Anixa and Jason.

TABLE OF CONTENTS

Chapter 1

1. INTRODUCTION 1

2. HISTOLOGY OF THE SMALL INTESTINE 1

A. Layers of the Intestinal Mucosa 2

B. Epithelial Crypts and Villi 3

C. Undifferentiated Crypt Cells 4

D. Small Intestine Disaccharidases 5

3. GROWTH FACTORS IN THE CHILD

A. Polyamines 6

B. Cortisol 10

C. Thyroxine 13

D. Epidermal Growth Factor 16

E. Insulin-Like Growth Factors 21

F. Growth Hormone 25

G. Possible Interaction Between Growth Hormone and Insulin-Like Growth Factor-I 29

4. GROWTH FACTORS IN HUMAN MILK AND THEIR ROLE IN INTESTINAL DEVELOPMENT 30

5. DIETARY FATTY ACIDS 34

Membrane Composition and Function 35

6. SUMMARY 40

Chapter 2

1. RATIONALE 41

2. HYPOTHESES 42

3. OBJECTIVES 43

Chapter 3

MATERIALS AND METHODS

1. Animals and Diets 44

2. Insulin-Like Growth Factor-1 Reconstitution 47

3. Removal of Small Intestine 47

4. Brush Border Membrane Isolation 47

5. Sucrase and Lactase Assays 48

6. Protein Determination 48

7. Lipid Analysis 48

A. Thin-Layer Chromatography 48

B. Methylation of Fatty Acids 49

C. Gas-Liquid Chromatography 49

8. Statistical Analysis 50

Chapter 4

RESULTS

1. Animal Characteristics

A. Gender 52

B. Body Weight 52

C. Intestinal Weight 52

D. Intestinal Length 54

2. Brush Border Membrane Protein Concentration 55

3. Sucrase Activity 55

4. Lactase Activity 56

A. Units per Gram of Protein 56

B. Units per Gram of Mucosal Weight 57

C. Units per Gram of Intestinal Weight 57

D. Units per Gram of Body Weight 57

5. Brush Border Membrane Phospholipid Composition

A. Phosphatidylcholine 58

B. Phosphatidylethanolamine 60

C. Phosphatidylserine 63

D. Phosphatidylinositol 65

E. Effect of IGF-I on Phospholipid Fatty Acid Composition 66

F. Relationship Between Lactase Activity and Phospholipid Fatty Acids 66

Chapter 5

1. DISCUSSION 68

2. SUMMARY 78

References 79

LIST OF TABLES

1.1 IGF-I Content of Ingested Mature Rat Milk 34

3.1 Basal Diet Composition per Kilogram of Diet 45

3.2 Fatty Acid Composition of Diets Fed 46

4.1 Effect of Diet on Pup Body Weight 53

4.2 Effect of Diet on Jejunal and Ileal Brush Border Membrane Lactase Activity 58

4.3 Effect of Diet on the Fatty Acid Composition of Brush Border Membrane Phosphatidylcholine (%w/w) 59

4.4 Fatty Acid Composition of Phosphatidylcholine (%w/w) in Jejunum and Ileum 60

4.5 Effect of Diet on the Fatty Acid Composition of Phosphatidylcholine (%w/w) in Jejunum and Ileum 60

4.6 Effect of Diet on the Fatty Acid Composition of Brush Border Membrane Phosphatidylethanolamine (%w/w) 61

4.7 Fatty Acid Composition of Phosphatidylethanolamine (%w/w) in Jejunum and Ileum 62

4.8 Effect of diet on the Fatty Acid Composition of Phosphatidylethanolamine (%w/w) in Jejunum and Ileum 62

4.9 Effect of IGF-I and Diet on Phosphatidylethanolamine C22:6(3) (%w/w) . 63

4.10 Effect of Diet on the Fatty Acid Composition of Brush Border Membrane Phosphatidylserine (%w/w) 64

4.11 Fatty Acid Composition of Phosphatidylserine (%w/w) in Jejunum and Ileum 65

4.12 Effect of Diet on the Fatty Acid Composition of Phosphatidylserine (%w/w) in Jejunum and Ileum 65

4.13 Effect of IGF-I on Phospholipid Fatty Acid Composition (%w/w) 66

LIST OF FIGURES

4.1 Effect of IGF-I Treatment on Intestinal Weight (% Body Weight) 53

4.2 Effect of Diet and IGF-I Treatment on Intestinal Length 54

4.3 Effect of Diet on Brush Border Membrane Protein Concentration 55

4.4 Effect of Diet on Brush Border Membrane Sucrase Activity 56

5.1 Relationship Between Lactase Activity and Phosphatidylcholine C20:4(6) Using Data From All Four Diets 76

5.2 Relationship Between Lactase Activity and Phosphatidylethanolamine C20:4(6) Using Data From All Four Diets 77

5.3 Relationship Between Lactase Activity and Phosphatidylcholine C22:6(3) Using Data From All Four Diets 77

LIST OF ABBREVIATIONS

AA	arachidonic acid
DFMO	D,L-α-difluoromethylornithine
DHA	docosahexaenoic acid
EGF	epidermal growth factor
GH	growth hormone
IgA	immunoglobulin A
IGFs	insulin-like growth factors
IGF-I	insulin-like growth factor-1
IGF-II	insulin-like growth factor-2
MCT	medium-chain triglyceride
PBS	phosphate buffered saline
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
PGE_2	prostaglandin E_2
TGF	transforming growth factor

Chapter 1.

1. INTRODUCTION

Growth hormones play an important role in the development of the gastrointestinal tract. Prior to birth, growth factors influence development through their presence in fetal and placental tissues and in amniotic fluid. Following birth, oral nutrition continues this developmental role by providing necessary growth factors and nutrients, such as essential fatty acids, through breast milk. It is hypothesized that the oral administration of insulin-like growth factor-I will enhance the precocious development of the suckling rat small intestine. Dietary intervention, resulting in fatty acid compositional changes in breast milk, may also influence small intestine ontogeny. A variety of growth factors which have been implicated as propagators of intestinal growth and development will be discussed.

2. HISTOLOGY OF THE SMALL INTESTINE

The small intestine is coiled within the abdominal cavity and extends from the pyloric orifice of the stomach to the ileocecal junction where the large intestine begins. In the human, the small intestine is twenty to twenty three feet in length and is divided into three sections. These sections include the duodenum, the jejunum and the ileum (Madara and Trier, 1994).

The first of these sections, the duodenum, is approximately ten to twelve inches long. This portion contains no mesentary and is therefore fixed to the wall of the posterior abdomen. The next seven and a half to nine feet of bowel

comprises the jejunum with the remaining eleven and a half to thirteen feet being known as the ileum. The mesentary or omenta, a layer of loose areolar tissue (Leeson and Leeson, 1970), suspends the jejunum and ileum from the posterior abdominal wall. The terminal ileum is not suspended but found to be attached to the posterior abdomen as is the duodenum (Madara and Trier, 1994).

A. Layers of the Intestinal Mucosa

The mucosa of the small intestine is comprised of three distinct layers (Antonioli and Madara, 1992). The deepest portion of the mucosa is known as the muscularis mucosa. This continuous sheet of smooth muscle is three to ten cells in depth and separates the intestinal mucosa from the submucosa. The function of the muscularis mucosa is unclear at present, but it has been speculated that the muscular nature of this layer may contribute to the movement of villi through the contractile properties it possesses (Madara and Trier, 1994; Antonioli and Madara, 1992).

A continuous connective tissue space regarded as the lamina propria lies above the muscularis mucosa. The connective tissue present in this space extends between intestinal glands and into the cores of villi and surrounds the crypt epithelium (Leeson and Leeson, 1970). In the human lamina propria of the small intestine the majority of the cells are mononuclear including plasma cells, T and B lymphocytes and macrophages (Madara and Trier, 1994; Goldman, 1992; Keren, 1992). Also present, but smaller in quantities, are eosinophils, mast cells, fibroblasts and myofibroblasts. The lamina propria provides structural support for epithelial cells located directly above it. It is also very rich in blood vessels which

provide the epithelium with nourishment (Madara and Trier, 1994).

Above the lamina propria lies a thin basement membrane. This membrane contains fibroblasts on its abluminal surface which aid in epithelial functions such as water and ion transport as well as differentiation. Directly above this basement membrane and in contact with the gut lumen is the epithelium. It is formed by a sheet of single epithelial cells and blankets the villi and their surrounding crypts (Antonioli and Madara, 1992).

B. Epithelial Crypts and Villi

The crypt epithelium is made up of undifferentiated cells, a variety of differentiated endocrine epithelial cells, paneth cells with large secretory granules, goblet cells that secrete mucus and rare tuft cells (Madara and Trier, 1994). The crypt is the site at which cell renewal occurs (Antonioli and Madara, 1992), primarily for undifferentiated and young goblet cells (Madara and Trier, 1994). The major role of the crypt is that of secretion. Exocrine secretion of goblet, paneth and undifferentiated cells into the crypt lumen takes place after their production in the base of the crypt as well as the secretion of water and ions. Endocrine secretion of endocrine epithelial cells into the lamina propria is also performed by these crypts (Madara and Trier, 1994).

Epithelial villi increase the surface area of the small intestine dramatically thereby enhancing the absorptive area. In individuals with diseases that disturb mucosal function it is often observed that the normal villus architecture is altered substantially reducing the ability of the small intestine to take up nutrients (Madara and Trier, 1994). These finger or leaf-like projections contain a central core of

lamina propria and are blanketed with epithelial cells (Leeson and Leeson, 1970). Within this epithelial lining exist many cell types such as enterocytes, goblet cells which secrete mucus, endocrine epithelial cells, rare tuft cells and cup cells (Antonioli and Madara, 1992). Intraepithelial T cells, primarily $CD8^+$, α/β T-cell receptors, and CD45RO cells, reside between villi epithelial cells (Rubin, 1995) along their basolateral side (Blumberg and Stenson, 1995). The most prominent function of the villus epithelium is nutrient absorption as the villus itself is the site of terminal digestion and absorption of nutrients and absorption of ions and water (Antonioli and Madara, 1992).

C. Undifferentiated Crypt Cells

Undifferentiated crypt cells are a major component of each small intestinal crypt (Antonioli and Madara, 1992). These cells are monoclonal in nature and are derived from a single stem cell. Sparse and short, undifferentiated crypt cells often contain irregular microvilli and a less well developed terminal web. In the mature crypt four to sixteen anchored stem cells are found near the base of the crypt. These stem cells serve as precursors for other intestinal epithelial cells including undifferentiated crypt cells as well as differentiated, absorptive, goblet, endocrine and paneth cells. A structural feature to differentiate these stem cells from other undifferentiated cells found in the crypt is not known at present. It is known that these cells do not absorb lipids or contain the necessary functional integral membrane proteins for terminal digestion and absorption of nutrients (Madara and Trier, 1994; Antonioli and Madara, 1992). They do however synthesize an IgA

receptor and place it on the lateral plasma membrane (Madara and Trier, 1994). IgA, produced by lamina propria plasma cells, is taken up into the intestinal epithelium (Blumberg and Stenson, 1995; Rubin, 1995). Once IgA has bound to the stem cell produced IgA receptor the undifferentiated crypt cells internalize this complex and transports it to the apical membrane where the ligand-receptor complex is chemically extruded and released into the lumen (Madara and Trier, 1994; Antonioli and Madara, 1992; Keren, 1992).

D. Small Intestine Disaccharidases

Dietary carbohydrates include the disaccharides sucrose and lactose, monosaccharides, glucose and fructose, and the plant starches amylose and amylopectin (Carethers, 1996; Guyton and Hall, 1996). Starch is digested to its respective disaccharide forms, maltose and maltotriose, by salivary and pancreatic α-amylases (Guyton and Hall, 1996; Noren, 1986). Maltose, lactose, and sucrose must be further hydrolysed into there respective monosaccharide components prior to take up by intestinal cell transporters (Guyton and Hall, 1996; Noren, 1986; Koldovsky, 1981). Intestinal disaccharidase enzymes, maltase, sucrase, and lactase, fulfill this final digestion of dietary carbohydrate molecules.

Prenatally, brush border membranes of rat enterocytes express significant levels of lactase activity. These levels increase substantially prior to birth in preparation for large concentrations of lactose provided in maternal milk (Olanrewaju *et al*, 1996; Sangild *et al.*, 1995; Henning, 1985). Increased lactase activity in the fetus is believed to be caused by an increase in circulating lactose in

the mother prior to birth (Koldovsky, 1981). Injection of lactose into the amniotic sac of fetal rats results in increased lactase activity (Koldovsky, 1981). Lactase activity levels remain high throughout the suckling period (Olanrewaju *et al.*, 1996; Kaouass *et al.*, 1994). Sucrase activity is absent during the fetal period and remains low or barely detectable until weaning commences (Ménard and Calvert, 1991; Koldovsky, 1981). At the time of weaning, 15 to 21 days postnatally, dramatic changes in sucrase and lactase expression occur. Sucrase levels dramatically increase while lactase activity declines rapidly (Olanrewaju *et al.*, 1996; Marti and Fernandez-Otero, 1994; Wild *et al.*, 1993; Liu *et al.*, 1992; Kretchmer, 1985). It is believed that these changes are genetically programmed, although hormonal and dietary factors have been shown to influence the timing of these abrupt changes (Kaouass *et al*, 1996; Wang and Xu, 1996; Wild *et al.*, 1993; Georges *et al.*, 1990;). Thus, in a variety of studies sucrase and lactase enzyme activities have been utilized as indicators of intestinal maturity (Ohneda *et al.*, 1997; Kaouass *et al.*, 1996; Olanrewaju *et al.*, 1996; Wang and Xu *et al.*, 1996; Kaouass *et al.*, 1994; Marti and Fernandez-Otero, 1994; Georges *et al.*, 1990; Young *et al.*, 1990).

3. GROWTH FACTORS IN THE CHILD

A. Polyamines

The polyamines putrescine, spermidine and spermine are ubiquitous. All nucleated prokaryotic and eukaryotic cells containing the amino acid ornithine are capable of polyamine synthesis (Conteas *et al.*, 1991; Jenkins and Thompson,

1994). Intracellular polyamines present in the gastrointestinal mucosa are made available through two processes. First, the decarboxylation of ornithine by the ornithine decarboxylase enzyme occurs, yielding putrescine. The ornithine decarboxylase enzyme is considered the rate-limiting enzyme in polyamine metabolism (Wang *et al.*, 1994). Propylamino groups, resulting from the decarboxylation of methionine, are added to putrescine to form both spermidine and spermine, respectively (Conteas *et al.*, 1991). Second, polyamines are absorbed from the gut lumen and accumulate in the intestinal epithelial cells (Wang *et al.*, 1994). Polyamines in the intestinal luminal contents enter the lumen through ingested food or are synthesized by intra luminal bacteria. The intra luminal bacteria capable of polyamine synthesis are found only in the ileum, cecum and the colon. Polyamines from gastrointestinal secretions and polyamines present in sloughed cells are also made available to the lumen (Johnson and McCormack, 1994).

Evidence exists to suggest that polyamines play a direct role in the maturation of the intestinal mucosa. Studies using D,L-α-difluoromethylornithine (DFMO), a specific inhibitor of ornithine decarboxylase, have demonstrated retardation of intestinal mucosal growth (Johnson and McCormack, 1994). In addition to the inhibition of ornithine decarboxylase activity, DFMO also results in a decline in cellular polyamine content. This decline often results in an inhibition or complete cessation of cell and tissue growth (Conteas *et al.*, 1991). Small intestinal mucosal cell proliferation responses can be prevented when DFMO is administered

under conditions including jejunectomy and fasting-refeeding situations (Jenkins and Thompson, 1994). Johnson and McCormack observed swelling of the Golgi apparatus in small intestinal epithelial cells upon the development of polyamine deficiency as a result of DFMO treatment. As well, DFMO resulted in a decrease in the amount of Golgi apparatus. It was concluded that this subcellular organelle was the most susceptible to polyamine deficiency (Johnson and McCormack, 1994).

Polyamines are required for normal cell growth and differentiation (Johnson and McCormack, 1994). Cell proliferation depends upon polyamine exposure to dividing cells in the small intestinal mucosa (McCormack and Johnson, 1991). Jenkins and Thompson propose that an increase in ornithine decarboxylase activity, and resulting increases in polyamine levels, only trigger an increase in mucosal growth rather than maintain cell proliferation levels. This hypothesis originates from data showing that little effect on ileal mucosal weight is observed in normally fed rats receiving DFMO (Jenkins and Thompson, 1994).

Enzymatic changes in the developing intestine correlate with alterations in the levels of ornithine decarboxylase and polyamines (Kaouass *et al.*, 1994). Corticosteroid and thyroid hormones are known to be major factors in changing disaccharidase levels (please refer to sections 3B and 3C, respectively). Studies examining the effects of polyamines on these changes have been performed (Kaouass *et al.*, 1994; Wild *et al.*, 1993; Shimizu *et al.*, 1993; Conteas *et al.*, 1991). In the rat, ornithine decarboxylase activity increases approximately 10-to-20 fold during the third week of life prior to weaning (Johnson and McCormack, 1994; Conteas *et al.*, 1991). This increase precedes the appearance of either the

increased number of mucosal cells or increases in mucosal enzyme activities (Yang *et al.*, 1984; Conteas *et al.*, 1991). Following mucosal maturation, both ornithine decarboxylase and polyamine levels decline to low basal levels (Conteas *et al.*, 1991). Conteas and colleagues (1991) found delayed histological and enzymatic maturation of the intestine when DFMO was administered to rat pups between postnatal days 14 and 24. Levels of diamine oxidase and mucosal disaccharidases did not reach normal levels until day 32 in treatment animals (Conteas *et al.*, 1991). Wild and coworkers (1993) examined changes in lactase and sucrase activities upon oral administration of spermidine at days 7-9. A significant and premature decline in lactase activity was observed by day 10. Sucrase activity appeared prematurely, and reached levels equivalent to mature animals by postnatal day 10 (Wild *et al.*, 1993). Orally administered spermine induces maturation of lactase activity in 3-day-old rats (Shimizu *et al.*, 1993). When spermine is administered orally, no changes were observed in maltase and sucrase activities in 11-day-old rats (Kaouass *et al.*, 1994). Lactase activity underwent a slight decrease in the distal portion of the small intestine. When spermine is administered surgically into the distal intestine, sucrase and maltase were greatly enhanced throughout the entire small intestine. Lactase activity remained unchanged in the proximal portion of the small intestine. A decline in lactase activity occurred in the distal portion (Kaouass *et al.*, 1994).

Polyamines possess the ability to stabilize membranes. By binding to the polar head groups of the membrane, polyamines protect the membranes against

lipid peroxidation. Polyamines play a role in regulating membrane-bound enzymes, transporting ions and metabolites, calcium homeostasis, polyphosphoinositide metabolism, protein kinase C, phospholipids and membrane fusion. These actions appear to be tissue specific. To date, these parameters of polyamine function have not been examined in the gastrointestinal tract (Johnson and McCormack, 1994).

B. Cortisol

Cortisol is the primary glucocorticoid found in humans. Cortisol accounts for approximately 95 percent of all glucocorticoid activity. The adrenal cortex produces cortisol. Being a steroid hormone, cortisol is derived from cholesterol absorbed directly from the circulating blood into the adrenal cortex. Specific enzymes catalyze the steps through which cortisol is formed. Essentially all of these steps occur within the mitochondria and the endoplasmic reticulum of the adrenal cortex cells. Once formed, the cortisol is transported through the extracellular fluid compartment either bound with a globulin or in a free form. Cortisol-binding globulin or transcortin, and to a lesser extent albumin, normally bind approximately 94 percent of the formed cortisol. The remaining six percent enters the blood stream in a free form. Upon release, cortisol becomes bound to target tissues. Unfixed cortisol is degraded primarily in the lumen within one or two hours following release. The degraded form of cortisol is conjugated to form glucuronides and to a lesser extent sulfates. Seventy-five percent of the metabolites are excreted in the urine, with the remainder being excreted in bile and the feces (Guyton and Hall, 1996).

The most studied metabolic effect of cortisol is its ability to stimulate liver gluconeogenesis. All enzymes required for amino acid conversion to glucose in the

liver are elevated by cortisol. Amino acids in extrahepatic tissues, primarily muscles, are mobilized by the presence of cortisol. Glucose utilization by cells elsewhere in the body decreases moderately in response to cortisol, thereby resulting in elevated glucose levels (Guyton and Hall, 1996).

Cortisol plays a role in the development of the small intestine. Digestive hydrolase expression is influenced by changes in glucocorticoid concentrations at weaning in the rat (Nsi-Emvo *et al.*, 1996). Release of corticosterone, a hormone similar to cortisol but less potent, has been observed to be one of the main factors in small intestine maturation (Marti and Fernandez-Otero, 1994; McDonald and Henning, 1992). Indirect evidence shows that cortisol plays a regulatory role in the developing human intestine. A marked increase in gestational cortisol levels of the fetus have been correlated with an increase in brush border membrane disaccharidase activities in the human fetus. Also, when glucocorticoids are exogenously administered to pregnant mothers prior to preterm delivery, a decrease in the incidence of necrotizing enterocolitis is observed in the preterm infant (Sangild *et al.*, 1995; Sheard and Walker, 1988).

Due to ethical considerations, most studies of change in postnatal disaccharidase activities have been performed in the rodent or swine model. Studies in the rat have shown that major alterations in brush border membrane enzyme activity occur during the second week in preparation for ingestion of solid food and the third week during weaning (Nsi-Emvo *et al.*, 1996; Marti and Fernandez-Otero, 1994; Needleman *et al.*, 1993; McDonald and Henning, 1992).

Postnatal sucrase, sucrase-isomaltase and lactase expression are enhanced by the presence of glucocorticoids in the first two postnatal weeks. An abrupt decline of intestinal lactase activity is observed late in the third week post partum (Yeh *et al.*, 1991a; Koldovsky, 1981). Following premature weaning an increase in sucrase activity is absent in adrenalectomized suckling rats (Koldovsky, 1981). This supports the suggestion of the requirement of glucocorticoids for this event (Nsi-Emvo *et al.*, 1996). Administration of exogenous cortisol to suckling animals elongates the intestinal microvilli and increase certain enzymes, including sucrase and alkaline phosphatase, in brush-border membranes (Sheard and Walker, 1988). Circulating corticosterone levels increase following food deprivation in rat pups. Premature expression of sucrase-isomaltase appeared to be triggered by this stress-induced rise in corticosterone levels (Nsi-Emvo *et al.*, 1996; Koldovsky, 1981).

The direct mechanism through which glucocorticoids influence disaccharidase enzyme activities is unknown. It is speculated that cortisol and other glucocorticoids do not act independently, but are involved with an array of other factors. Marti and colleagues (1994) observed an increase in circulating corticosterone levels upon subcutaneous administration of prostaglandin E_2 (PGE_2). Activities of sucrase, aminopeptidases and maltase throughout the small intestine and lactase in the distal portion were found to increase. It is speculated that the increased activity of these enzymes resulted from the ability of PGE_2 to increase corticosterone levels by up to 84 percent as compared to control rats (Marti and Fernandez-Otero, 1994). Under conditions of stress, such as food deprivation,

isolation or exposure to cold, levels of corticosterone increase (Guyton and Hall, 1996; Opper and Heizer, 1995). Nsi-Emvo and colleagues (1996) observed a parallel increase of corticosterone levels with that of ornithine decarboxylase activity and mRNA in preweaned intestine. An increase in mucosal polyamine content followed these increases. It was concluded that the increase in ornithine decarboxylase and subsequent polyamine levels, due to the rise in glucocorticoids, mediated the premature expression of sucrase-isomaltase mRNA and sucrase activity (Nsi-Emvo *et al.*, 1996). Thyroxine has a major influence over circulating levels of exogenous and endogenous glucocorticoids (Podolsky and Babyatsky, 1995; Menard and Calvert, 1991). Thyroxine increases the production of corticosteroid-binding globulin (McDonald and Henning, 1992). When corticosteroid-binding globulin concentrations are increased more corticosterone becomes bound, thus decreasing corticosterone volume of distribution and metabolic clearance rate (McDonald and Henning, 1992). As a result increased concentrations of circulating corticosterone result (Needleman *et al.*, 1993; McDonald and Henning, 1992).

C. Thyroxine

Thyroxine is a derivative of the amino acid tyrosine. The synthesis of thyroxine occurs in the thyroid gland. Tyrosine is iodinized, resulting in monoiodotyrosine and diiodotyrosine residues which become coupled with one another. The major product of the coupled residues is thyroxine. Triiodothyronine is also produced. Both iodinated tyrosine derivatives are stored attached to

thyroglobulin molecules (Guyton and Hall, 1996). Thyroid-stimulating hormone is the primary controller of thyroxine and triiodothyronine secretion. Thyroxine and triiodothyronine are cleaved from the thyroglobulin molecule prior to release. Approximately 93 percent of the thyroid hormone released is thyroxine, with the remaining 7 percent being present in the triiodothyronine form. Following release from the thyroid gland most of the thyroxine is deiodinated to form additional triiodothyronine. Therefore, the hormone reaching tissues is usually triiodothyronine (Guyton and Hall, 1996). Thyroxine and triiodothyronine share the same functions: triiodothyronine is four times as potent as thyroxine but is found in smaller quantities in the blood and persists for a shorter duration (Guyton and Hall, 1996).

Circulating levels of thyroxine increase during the second postnatal week in the rat (McDonald and Henning, 1992). Exogenous administration of thyroxine to intact animals results in a premature decline in intestinal lactase and a large increase in sucrase, maltase as well as glycoamylase activities (Menard and Calvert, 1991). Administration of thyroxine also results in increased plasma corticosterone levels. When corticosterone levels are not allowed to increase, thyroxine administration during the first two postnatal weeks has no effect on sucrase and maltase levels (Menard and Calvert, 1991). Yeh and colleagues (1991b) examined the effects of thyroxine and cortisone, alone and in combination, on the mRNA and activity of lactase, sucrase-isomaltase and sucrase in suckling rats: thyroxine in combination with cortisone elevates the abundance of sucrase-isomaltase mRNA and sucrase-isomaltase protein content. Lactase activity increases in response to cortisone administration in nine day old sucklings.

Thyroxine administration alone had no effect on the lactase activity at this age (Yeh *et al.*, 1991b). When thyroxine was combined with cortisone, the increase in jejunal lactase activity ceased (Podolsky and Babyatsky, 1995; Yeh *et al.*, 1991a,b). Lactase mRNA levels remained high, although the activity was decreased (Yeh *et al.*, 1991b). Sucrase activity in the jejunum, which was negligible or undetectable at nine days of age, was elevated significantly by the administration of cortisone. This rise was influenced further by the combination of cortisone with thyroxine (Yeh *et al.*, 1991b).

Premature weaning of rat pups results in increased sucrase activity and a delayed reduction of lactase activity (Shinder *et al.*, 1995). Lower levels of circulating thyroxine are observed in the premature weaning state. When pups are injected with endogenous thyroxine, lactase reduction commences (Shinder *et al.*, 1995). Exposure to cold (which enhances corticosterone release) and thyroxine administration following preweaning allow for recovery of body and intestinal weights and digestive enzyme activities (Shinder *et al.*, 1995).

Thyroxine plays an important role in governing changes in intestinal lactase during the weaning period (Liu *et al.*, 1992). Hypophysectomy results in elevated lactase activity following the weaning period in rats (Castillo *et al.*, 1991). The decline in lactase activity following administration of either thyroxine or triiodothyronine to these hypophysectomized animals presents evidence for the direct involvement of thyroid hormones in intestinal adaptation and maturation (Hodin *et al.*, 1994; Castillo *et al.*, 1991).

D. Epidermal Growth Factor

Epidermal growth factor (EGF) was first discovered in 1960 by Cohen. EGF is a 6043 Da polypeptide chain comprised of 53 amino acids (Santer *et al.*, 1993). EGF is a prototypic member of a family consisting of at least seven different peptides. These peptides, or EGF molecules, are defined by three distinct properties (Podolsky, 1994). First, they must be able to bind with the EGF receptor. Second, they must be capable of mimicking the biological activities of EGF. Third, an EGF motif characterised by six half-cysteine residues must be present within a 50 to 60 residue primary structure. Each EGF motif peptide must contain these three properties. The level of sequence homology within the EGF family is approximately 20 percent (Podolsky, 1994). Transforming growth factor-α (TGF-α), an EGF analogue with 30 to 40 percent amino acid homology, acts in a similar fashion to EGF. The TGF-α peptide has been proposed to be an embryonic form of EGF which is inappropriately expressed in neoplasia (Santer *et al.*, 1993). EGF is primarily produced as a prepro-EGF-containing protein. It is believed that this 1200 residue precursor contains the mature EGF. As well, eight other EGF-like domains are present, and proteolytic cleavage of this residue results in the release of these domains and the EGF fraction (Podolsky, 1994).

The highest concentrations of EGF are found in the salivary glands and in the kidneys (Karnes, 1994). Various tissues contain EGF such as the Brunner's glands of the duodenum, the pancreas, submandibular glands, the thyroid gland and the Paneth cells of the small intestine (Karnes, 1994; Koldovsky *et al*, 1991c). EGF is

present in body fluids including saliva, urine, amniotic fluid, semen, bile secretions, pancreatic and duodenal juices, as well as in breast milk (Karnes, 1994; Hardin *et al.*, 1993; Opleta-Madsen *et al.*, 1991b). Secretion from the salivary glands and the pancreas are under neurohormonal control. Sham feeding and pentagastrin are noted to induce EGF from these tissues. Administration of atropine and somatostatin inhibit secretion from these sources (Karnes, 1994).

Breast milk contains high levels of various growth promoting peptides (Shams, 1994; Koldovsky and Thornburg, 1987). The growth promoting activities have been considered to be mostly attributable to EGF, insulin-like growth factors and transforming growth factor-α (Kanda *et al.*, 1994). The presence of considerable concentrations of EGF in breast milk has led to the speculation that EGF may play a role in mediating postpartum gastrointestinal growth and development (Podolsky, 1994; Hardin *et al.*, 1993; Opleta-Madsen *et al.*, 1991b). Levels of EGF and EGF mRNA are absent or detectable at very low levels in fetal mouse tissues (Fisher and Lakshmanan, 1990). The significant amounts of EGF present in breast milk may be the major source of this peptide for suckling mammals (Koldovsky *et al.*, 1991a). Absence of EGF and other milk-borne substances may be accountable for necrotizing enterocolitis in premature infants fed commercially prepared formulas (Koldovsky *et al.*, 1991c).

Human colostrum contains between 20 to 438 ng/ml of EGF, while mature milk has lower levels of EGF ranging from 20 to 110 ng/ml (Santer *et al.*, 1993). Milk obtained from mice and rats contains an EGF concentration of 50 to 500 ng/ml

and 30 to 50 ng/ml, respectively (Koldovsky *et al.*, 1991c). The relatively low EGF content in gut tissues observed at birth progressively increase in the suckling rat (Fisher and Lakshmanan, 1990). This observation suggests that gastrointestinal EGF content is largely influenced by milk intake (Koldovsky *et al*, 1991a). When suckling rats are fasted, a considerable decline in mucosal EGF is observed (Koldovsky *et al.*, 1991b). Fisher and Lakshmanan (1990) observed dramatically accelerated synthesis of EGF during the early neonatal period in the mouse. Feeding was found to markedly increase these concentrations. When EGF antiserum was injected subcutaneously, on days one through nine of life, weight gain decreased by 40 percent and eye opening, ear opening, tooth eruption and duodenal Paneth cell granulation were delayed (Fisher and Lakshmanan, 1990). EGF is also important during pregnancy. Removal of the salivary gland (thus preventing increases in EGF) caused a 50 percent increase in the abortion rate (Fisher and Lakshmanan, 1990). Mice which completed their pregnancies delivered pups with crown-rump lengths lower than those observed in the control groups (Fisher and Lakshmanan, 1990). Subcutaneous administration of EGF antiserum to mice without salivary glands resulted in a further increased abortion rate. Exogenous EGF delivered subcutaneously to these animals improved the outcome of their pregnancies (Fisher and Lakshmanan, 1990). EGF is an acid-stable peptide (Hardin *et al.*, 1993), therefore very little degradation of EGF occurs in the gastrointestinal environment of suckling animals allowing these peptides to be delivered in an active form to peripheral organs (Koldovsky *et al.*, 1991c). When

animals are fasted or fed parenterally, the majority of EGF is rapidly digested in the lumen to inactive forms (Marchbank *et al.*, 1995). This digestion can be prevented by co-administration of protease inhibitors, and this combination has been recommended for reducing intestinal atrophy caused by parenteral feeding (Marchbank *et al.*, 1995).

EGF has a trophic effect on intestinal epithelial cells (Menard and Calvert, 1992; Goodlad *et al.*, 1991). Maturation is accelerated, cellular proliferation is stimulated and gastric secretion is modulated by its ability to inhibit the release of HCl by parietal cells (Weaver and Walker, 1988). Prostaglandin synthesis is modulated as well, through effects on cyclooxygenase and lipoxygenase enzymes (Weaver and Walker, 1988). The EGF receptor phosphorylates many proteins *in vitro* (Podolsky, 1994; Opleta-Madsen *et al.*, 1991a). Lipocortin I may be possibly the first substrate phosphorylated by the activated EGF receptor (Podolsky, 1994). Through this phosphorylation phospholipase activity is regulated, since the generation of mediators of the phosphoinositide-signalling cascade and various arachidonic acid metabolites are controlled through this protein. Phospholipase A_2 is inhibited in the presence of Lipocortin I, and thus the generation of arachidonate is inhibited (Podolsky, 1994). Potential anti-inflammatory activities in the gastrointestinal tract may be related to the action of the EGF receptor on these metabolites (Podolsky, 1994). For example, phospholipase-C and diacylglycerol are phosphorylated by the EGF receptor causing an up regulation of the phosphoinositide-signalling cascade (Podolsky, 1994). This action results in

increased levels of intracellular calcium and protein kinase C (Podolsky, 1994).

The growth and development of organs of epithelial and mesothelial origin are modulated by EGF. Skin, teeth, lungs, mammary glands, ovaries, kidneys, liver, pancreas and the entire gastrointestinal tract are affected. Acid secretion is inhibited, and angiogenesis is stimulated by EGF. Smooth muscle contraction, wound epithelialization and stimulation of various hormones are influenced by the presence of this growth hormone. In the gastrointestinal tract of fetal and suckling rodents, EGF enhances intestinal enzyme activity and increases calcium transport (Karnes, 1994). Protein and DNA synthesis in the small intestine is stimulated by EGF (Menard and Calvert, 1992; Koldovsky, 1981). Following subcutaneous injection of EGF, brush border membrane enzymes including sucrase, appear prematurely (Majumdar, 1991). Ingestion or injection of EGFs have profound effects on the immature gut. Gastric and intestinal weights are increased. Intestinal DNA and RNA contents increase. Calcium binding protein levels and calcium transport are stimulated. An increase in the number of vitamin D receptors is observed (Fisher and Lakshmanan, 1990). Activities of enterocyte ornithine decarboxylase, trehalase, lactase, glucoamylase and alkaline phosphatase increase (Podolsky, 1994; Fisher and Lakshmanan, 1990). Microvillous surface area increases with intra luminal EGF administration (Hardin *et al.*, 1993). This increase is dependent upon extracellular calcium, and is suggested to be due to the redistribution of existing intracellular plasma membrane (Hardin *et al.*, 1993). Increases in the absorption of water, sodium, calcium and glucose are linked to the increase in microvillous surface area (Hardin *et al.*, 1993; Opleta-Madsen *et al.*,

1991b). A significant decrease in brush border membrane vesicle cholesterol content and in the cholesterol-to-phospholipid ratio occur (Opleta-Madsen, 1991b). The phosphatidylethanolamine-to-phosphatidylcholine and the sphingomyelin-to-phosphatidylcholine ratios decrease significantly in brush border membrane vesicles (Opleta-Madsen, 1991b). It is believed that these decreases in the relative abundance of phospholipid subclasses attribute to the increase in membrane fluidity (Opleta-Madsen *et al.*, 1991b).

E. Insulin-Like Growth Factors

Insulin-like Growth Factors (IGFs), or somatomedins, are peptide growth hormones structurally related to pro-insulin. The normal growth and development of vertebrates is dependent upon IGF-I and IGF-II (LeRoith and Roberts, 1994). The liver is the major source of endocrine IGF-I (Quadros *et al.*, 1994). Paracrine and autocrine IGF-I are synthesized in most extrahepatic tissues (Olanrewaju *et al.*, 1996; LeRoith and Roberts, 1994; Quadros *et al.*, 1994). These extrahepatic somatomedins function as regulators in their source tissue (LeRoith and Roberts, 1994). Tissues are unable to store IGF-I, and will produce it upon requirement (Hatton *et al.*, 1993).

Growth hormone, a 191 amino acid protein, is secreted by the anterior pituitary gland (Shulman *et al.*, 1992). Postnatally, growth hormone acts as a major regulator of IGF-I synthesis and secretion (Fu *et al.*, 1991), although during embryonic development, and during a short period postnatally, IGF-I secretion appears to be growth hormone independent (Baker *et al.*, 1993). At weaning, the

hepatic production of IGF-I becomes growth hormone-dependent (Ulshen *et al.*, 1993). IGF-I is synthesized in a growth hormone-dependent manner, and mediates or influences many of the growth promoting effects of growth hormone (Ulshen *et al.*, 1993). The general action of IGF-I and growth hormone is the promotion of cell growth in tissues (Fu *et al.*, 1991).

Fetal tissues express high levels of IGF-II. IGF-II is found to play a paramount role in the overall growth and development of fetal tissues. Following birth, IGF-II levels decrease in some tissues. The role of IGF-II present in adult tissues is undetermined. Postnatal levels of IGF-I dramatically increase in most tissues (LeRoith and Roberts, 1994). Extrahepatic sources of somatomedins are not strongly regulated by growth hormone. In part, they are largely controlled by the hormones present in their source tissue (LeRoith and Roberts, 1994).

One of the major targets of IGF action is the gastrointestinal tract (Zeeh *et al.*, 1995). Effects of IGF include suppression of protein degradation at local and systemic levels, increased amino acid uptake, and the proliferation and differentiation of gastrointestinal cells (Zeeh *et al.*, 1995) and are potent inducers of ODC (Olanrewaju *et al.*, 1992). IGF activities are dependent upon the availability of free IGF concentrations (Hatton *et al.*, 1993). Specific IGF-I and IGF-II receptors have been located throughout the gastrointestinal tract (Zeeh *et al.*, 1995; Zhang *et al.*, 1995; Young *et al.*, 1990). In the small intestinal mucosa, IGF-I receptors are found in a decreasing crypt-villous gradient (Dvorak *et al.*, 1996; Ulshen *et al.*, 1993). Binding of free IGF-I to these receptors initiates the actions of IGF-I (Hatton

et al., 1993). Maximal IGF-I receptor density occurs in the suckling animal (Young *et al.*, 1990). Binding of IGF-I to receptors found on immature small intestinal tissue, such as that occurring prior to weaning, is two to six times greater than observed in adult tissue (Zumkeller, 1992; Young *et al.*, 1990).

Few studies have focused on the effects of IGF-I on suckling animals. The majority of studies involve pharmacological doses of IGF-I and their effects on resected or transplanted small intestine. Xu and colleagues (1994) examined the growth promoting effects of a commercial infant formula (SMA), supplemented with IGF-I, on the gastrointestinal tract of newborn pigs. Pancreatic tissue mass increased and proliferation of intestinal crypt cells was stimulated (Xu *et al.*, 1994). Suckling rat pups (d10-15) have demonstrated increases in brush border enzyme activities in response to daily oral and intra peritoneal IGF-I treatment. Maltase and lactase were found to increase. A premature rise in sucrase activity did not occur (Young *et al.*, 1990). Steeb and colleagues (1997) were able to observe age dependent differences in body weight in response to IGF-I injection. The body weight of rat pups receiving IGF-I on days 6 through 13 of life did not show significant changes when compared to their control littermates. Rat pups receiving IGF-I on days 12 through 19 of life, demonstrated heavier total body weights when compared to control pups. IGF-I - GH dependency, which occurs during weaning, accounts for this response. This suggests that GH is more effective in promoting changes in total body weight than IGF-I alone (Steeb *et al.*, 1997).

Trophic effects can be attained by infusing IGF into the ileum following gut

resection or transplantation. IGF-I plays a role in reducing protein catabolism, enhancing tissue healing, and increasing mucosal growth following injury. There is a marked increase in intestinal growth following dexamethasone treatment in rats when the procedure is performed in conjunction with exogenous IGF-I infusion (Zeeh *et al.*, 1995). Following small bowel transplantation in male Lewis rats, Zhang and colleagues (1995), provided continuous IGF-I infusion subcutaneously through an osmotic minipump. IGF-I at 2.4 mg/kg/day in 0.1 M of acetic acid was administered postoperatively to both small bowel transplantation and transection groups (Zhang *et al.*, 1995). IGF-I increased mucosal structural indices, intestinal weight and body weight of both transplanted and control transection groups (Zhang *et al.*, 1995). An increase in the absorption of both water and glucose supported the conclusion that structural changes in rat small intestine were complemented with improved jejunal function. The enhancement of absorptive functions may be of greater significance than the changes in mucosal density (Zhang *et al.*, 1995). Increased mucosal epithelial cell proliferation was found to be a direct result of IGF-I infusion in small bowel transplant groups. An increase in crypt depth and an improvement in villous height and surface area support this finding. Overall, an increase in both the replication of intestinal mucosal cells and the number of mature differentiated cells along the villus were observed as a direct result of exogenous IGF-I (Zhang *et al.*, 1995).

There is a synergistic relationship between IGF-I and EGF in the gastrointestinal tract. Enterocyte proliferation at a modest rate is observed when

IGF-I and epidermal growth factor are presented to cultured cells independently. These two growth factors are synergistic, promoting cell growth. In culture, EGF primes cells for IGF-I, which in turn act to increase the rate of proliferation. Thus, EGF acts as a competence factor, and IGF-I acts as a progression factor. (Duncan *et al.*, 1994).

Specific binding sites for IGF-I exist in the mucosa, muscularis mucosa, and muscularis propria of the rat colon (Ohneda *et al.*, 1997; Zeeh *et al.*, 1995). An increase of IGF-I binding sites were located in the muscularis propria of inflamed rat colon. The presence of these binding sites indicates an important role for IGF-I in inflammation mediation, tissue repair, and formation of granulation. IGF-I mediates growth promoting effects on various cells, including smooth muscle cells. Infiltration of IGF-I by increased numbers of binding proteins may play an active role in mediating the smooth muscle hyperplasia which is common in the inflamed bowel (Zeeh *et al.*, 1995).

F. Growth Hormone

Growth hormone (GH) is a 22 kDa protein containing 191 amino acids (Scanes and Campbell, 1995). Synthesized in the anterior pituitary gland, GH is essential for normal growth and development of all vertebrates (Guyton and Hall, 1996; Harvey, 1995). GH fosters growth throughout the entire body by influencing protein formation, cell multiplication and cell differentiation (Guyton and Hall, 1996). Somatotrophs in the anterior pituitary, house GH possibly as zinc-mediated dimers. The opposing actions of GH-releasing factor and somatostatin are the primary

controllers of GH release (Guyton and Hall, 1996; Clackson, 1994). The release of GH in secretory granules occurs in two ways: constitutive or regulated release. Constitutive release occurs in a calcium-independent manner whereby secretory vesicles are continually released into the plasma membrane (Carter-Su *et al.*, 1996). Regulated release is a calcium-dependent process (Carter-Su *et al.*, 1996). An increase in cytosolic calcium is required in order for intermittent exocytotic discharge of secretory granules (Carter-Su *et al.*, 1996; Harvey, 1995). An influx of calcium is accomplished by GH-releasing factor activation of the adenyl cyclase system (Carter-Su *et al.*, 1996). Levels of cAMP increase in response and calcium ion transport into the cell is increased (Carter-Su *et al.*, 1996). This short term action occurs within minutes of stimulation (Guyton and Hall, 1996). A long-term effect of the calcium influx is increased transcription of GH synthesis genes (Guyton and Hall, 1996).

GH synthesis and release is triggered by many physiological states. Circulating levels of GH increase in response to starvation, particularly severe protein malnutrition, hypoglycaemia, low circulating fatty acid concentrations, exercise, excitement, trauma and during the first two hours of deep sleep (reviewed by Guyton and Hall, 1996). Increases in GH levels result in a variety of metabolic effects. Protein synthesis rates are increased. Concentrations of amino acids are increased within all cells of the body. RNA translation is enhanced, resulting in increased ribosomal protein synthesis (Guyton and Hall, 1996; Christensen and Flyvbjerg, 1992; Herndon *et al.*, 1992). Reduction occurs in the breakdown of

protein. GH preferentially utilizes fat stores for energy, as opposed to carbohydrate and protein. The movement of large quantities of free fatty acids from adipose tissue increases the concentration of circulating free fatty acids. The free fatty acids are then utilized as energy by various cells, thus providing a protein-sparing effect (Guyton and Hall, 1996; Clackson, 1994). The rate of glucose utilization is decreased throughout the body in response to the presence of excess amounts of GH (Guyton and Hall, 1996; Herndon *et al.*, 1992). There is a decreased requirement of glucose for energy as free fatty acids are utilized. The excess glucose is taken up into cells where it is rapidly polymerized into glycogen and stored (Guyton and Hall, 1996). Cells become saturated with the excessive amounts of glucose present, and the overflow is deposited into the blood stream resulting in a condition regarded as "pituitary diabetes". The high circulating glucose concentrations signal the beta cells to secrete insulin. This diabetogenic effect of excessive GH administration leads to insulin insensitivity, and the large amounts of insulin generated to lower the plasma glucose levels result in beta cell exhaustion. When this occurs, diabetes mellitus develops (Guyton and Hall, 1996; Herndon *et al.*, 1992).

Specific receptors for GH have been demonstrated on epithelial cells in rat intestine (Shulman *et al.*, 1992). The GH receptor is a member of the cytokine receptor family, characterized by a single membrane-spanning domain with limited homology in the extracellular domain, four cysteine groups, a tryptophan, and a membrane-proximal WSXWS motif. This WSXWS motif is thought to play an

essential role in ligand binding (Carter-Su *et al.*, 1996). GH receptor genes have been observed throughout the digestive tract. *In situ* hybridization analysis using cDNA probes have identified a signal in the proliferative and differentiative zone of the small intestine and colon (Delehaye-Zervas *et al.*, 1994). The expression of GH receptor transcripts throughout the gastrointestinal tract imply a fetal and postnatal pleiotropic action of GH in the gut (Nagano *et al.*, 1995).

GH promotes intestinal water and electrolyte transport and enhances calcium absorption (Nagano *et al.*, 1995; Shulman *et al.*, 1992). Studies using hypophysectomized rats are able to demonstrate GH growth regulatory actions. Hypophysectomized rats display mucosal hypoplasia, a reduction in plasma gastrin, and declined calcium and phosphorous absorption (Nagano *et al.*, 1995; Shulman *et al.*, 1992). These effects are partially reversed upon subcutaneous injection of GH (Lund, 1994). Intestinal and gastric mucosal weight are restored, and the mitotic index of the duodenal crypt cells is increased (Nagano *et al.*, 1995; Olanrewaju *et al.*, 1992). A trophic degeneration of the intestinal mucosa in normally fed hypophysectomized rats implies that GH plays a role in the normal growth of the intestinal mucosa (Kissmeyer-Nielsen *et al.*, 1994). GH transgenic animals display intestinal hyperplasia and hypertrophy in the proximal bowel mucosa. Mucosal hypertrophy is observed throughout the entire bowel. These observations support an enterotropic role of both excess GH and insulin-like growth factor-1 in the bowel (Lund, 1994).

G. Possible Interaction Between Growth Hormone and Insulin-like Growth Factor-1

Growth hormone (GH) is thought to exert its growth promoting effects through stimulation of insulin-like growth factor-1 (IGF-I) production in the liver (Guyton and Hall, 1996; Scanes and Daughaday, 1995; Shulman *et al.*, 1992). The "somatomedin hypothesis" states that GH controls both the production and release of endogenous IGF-I. The IGF-I is then responsible for coupling GH release for somatic growth (Olanrewaju *et al.*, 1992). GH is important in regulating the levels of IGF-I in serum, and these resulting levels are known to influence endogenous GH secretion (Christensen and Flyvbjerg, 1992). Increased levels of IGF-I suppress GH secretion at the level of GH gene transcription (Harvey, 1995). Treatment with GH results in elevated levels of serum IGF-I (Herington, 1994). The local production of IGF-I in the gastrointestinal tract may also be stimulated directly by GH (Kissmeyer-Nielsen *et al.*, 1994). In transgenic animals over-expressing IGF-I, levels of GH are almost undetectable due to negative feedback control of IGF-I on the pituitary gland. These transgenic animals do not display bowel hypertrophy or hyperplasia. Absence of these conditions, which are evident in transgenic animals over-expressing GH, is speculated to be due to the GH deficient state of these animals (Lund, 1994). Extrahepatic IGF-I is less dependent on GH and is found to be more tissue specific. At the time of weaning, hepatic expression of IGF-I becomes GH dependent (Le Roith and Roberts, 1994). Intact animals receiving GH injections display proliferation and growth of cells (Podolsky and Babyatsky, 1995).

IGF-I is produced by the liver in response to increased GH levels. The half-life of GH is 20 minutes (Guyton and Hall, 1996). When injected, GH weakly attaches itself to plasma proteins and is circulated in the blood and released rapidly into tissues. The half-life of IGF-I is 20 hours, and can greatly prolong the growth promoting effects of GH secretion bursts (Guyton and Hall, 1996). The "dual-effector hypothesis" supports this theory of GH/IGF-I synergism. The theory states that GH commits stem cells to a particular differentiative pathway, and that the IGF-I mediates clonal expansion of these committed or differentiated stem cells following the elapse of GH activity (Lund, 1994).

4. GROWTH FACTORS IN HUMAN MILK AND THEIR ROLE IN INTESTINAL DEVELOPMENT

A pivotal role in gastrointestinal development may be played by the insulin-like growth factors found in milk or by a combination of IGF-I and -II with many known and unknown growth factors. Intestinal mucosal barrier development may be enhanced by the growth hormones found in human milk. An increased rate of cellular turnover may be responsible for this increase in mucosal barrier function. An increase in the infants' ability to protect itself from harmful antigens in the gut lumen at an earlier time may arise (Zumkeller *et al.*, 1992). Epidemiological studies have shown a possible link between children who were breast fed, and a decreased incidence of future disease (Goldman *et al.*, 1991) For example, in the USA, a study has shown that there is a decreased risk of developing type I diabetes mellitus

in subjects who were breast fed as infants. A Canadian study revealed that the development of chronic inflammatory diseases of the small bowel, such as Crohn's disease, are lower in children who were breast fed as infants (Goldman *et al.*, 1991). IGF-I is believed to be an important mediator of the intestines response to local inflammation (Zumkeller *et al.*, 1992). Therefore it is plausible that the gut may be able to build up defence mechanisms against these particular diseases in response to exposure of breast milk during infancy.

There may be a direct stimulatory effect of breast milk on intestinal growth (Ichiba *et al.*, 1992). Radioimmunoassay and radioreceptorassay techniques were used to determine concentrations of growth factors in human breast milk and infant formulas. Human milk from mothers of preterm and term infants were examined: the concentration of EGF was high in colostrum of both human milk groups (Ichiba *et al.*, 1992). A rapid decline in EGF concentration was observed, but this decline stabilized by day 4 of lactation. During lactation, the level of IGF-I increased gradually in both human milk groups. Concentrations of EGF and IGF-I in a commercial infant formula were both below a detectible level (Ichiba *et al.*, 1992). A bioassay of growth-promoting activity of EGF and IGF-I was examined. Observed radioactivity, from the incubation of intestinal cells with [^{3}H]-thymidine, was defined as the growth promoting activity. Cultured fetal intestinal cells were incubated with varying concentrations of human milk, commercial infant formula, and purified human EGF or IGF-I. Growth promoting activity was high in colostrum samples, decreasing gradually during lactation (Ichiba *et al.*, 1992). A dose-dependent

response in activity to human milk EGF and IGF-I was observed. The growth promoting activity of EGF correlated with its concentration, whereas IGF-I did not (Ichiba *et al.*, 1992). A much greater concentration of IGF-I than is present in human milk was required to obtain a response. Commercial infant formula produced no growth promoting activity on the intestinal cell line. Both EGF and IGF-I demonstrated growth-promoting activity in the human fetal intestinal cell line (Ichiba *et al.*, 1992).

Cellular proliferation and maturation due to EGF appears to be both dependent upon the time of exposure and the route of administration (Opleta-Madsen *et al.*, 1991b). When given orally, EGF has a trophic effect which is highest in the jejunum and decreases in the area approaching the distal ileum (Opleta-Madsen *et al.*, 1991b). Both the jejunum and ileum experience a trophic effect when EGF is administered systemically (Goodlad *et al.*, 1991).

Increased microvillus membrane fluidity in response to EGF administration is due to a decrease in the cholesterol:phospholipid ratio. The relative concentration of phosphatidylcholine in the ileum increases. This increase led to the reduction of the ratios phosphatidylethanolamine:phosphatidylcholine and sphingomyelin:phosphatidylcholine. When viewed by unit length of intestine, oral and intraperitoneal injection of EGF increase water, Na^+ and glucose absorption rates. When absorption is expressed per mg of DNA, a significant increase is observed only in the systemic EGF group. This indicates that the rate of transport per cell is increased, and an increase does not result solely from an enhanced

number of absorptive cells. During the postnatal period, both an increase in epithelial cell mass and the induction of cellular transport processes allow the small intestine to enhance its nutrient transport capacity (Opleta-Madsen *et al.*, 1991b).

Zumkeller and colleagues (1992) observed higher levels of both IGF-I and IGF-II in colostrum than in mature milk. The levels decreased as the milk "matured". IGF-I content in human colostrum ranges between 8 and 28 ng/ml, while mature milk levels are between 5 and 10 ng/ml (Zumkeller, 1992; Donovan *et al.*, 1991). Mature rat milk contains between 5 and 21 ng/ml **(Table 1.1)** (Donovan *et al*, 1991; Philipps *et al.*, 1990; Godbole *et al.*, 1981). The mitogenic potency of IGF-I in colostrum was found to be higher than that of IGF-I in mature milk. This higher level possibly aids the neonatal gut during the transitional stage of development following birth (Zumkeller, 1992). These increased levels would be of importance to the premature infant if adaptation is enhanced by this growth factor. The presence of IGF-I receptors in the intestinal epithelium suggests that IGF supplementation may be beneficial in accelerating the development of the immature gastrointestinal tract (Donovan *et al.*, 1991).

Oral IGF-I appears to be active in the infant gut. *In vitro* studies have shown the absorption of intact IGF-I across the colonic mucosa (Quadros *et al.*, 1994). Stimulation of growth in the intestine has been observed upon ingestion of colostrum and early milk. IGF binds to the proliferative intestinal cells rather than to differentiated enterocytes. This suggests that IGF may be an important growth factor during this period of development (Zumkeller *et al.*, 1992). Almost 80% of

[^{125}I] IGF-I in milk is absorbed by suckling rats (Philipps *et al.*, 1990). Thus, a large proportion of ingested IGF-I is not digested, and may therefore be of importance systemically to the newborn (Philipps *et al.*, 1990).

Table 1.1
IGF-I Content of Ingested Mature Rat Milk

Day of Life	Milk Ingested (ml / Day)	Milk IGF-I (ng / Day)
10	2.0±0.2	28±19
11	3.0±0.3	41±28
12	4.0±0.4	55±37
13	5.0±0.4	68±45

(Zumkeller, 1992; Donovan *et al.*, 1991; Philipps *et al.*, 1990; Godbole *et al.*, 1981)

Intra luminal IGF-I is a potent inducers of enterocyte cytosolic ornithine decarboxylase activity. There is a sufficient quantity of IGF-I in milk to produce growth effects on the gastrointestinal tract. Polyamine synthesis is induced by the presence of IGF-I. The local infusion of IGF-I into the gut lumen results in increased growth in the rat gastrointestinal mucosa (Olanrewaju *et al.*, 1992).

5. DIETARY FATTY ACIDS

Unsaturated fatty acids of the ω9, ω7 ,ω6, and ω3 family can be elongated and desaturated utilizing the same elongase and desaturase enzymes (Peck, 1994). The ω6 and ω3 fatty acids, primarily linoleic (C18:2(6)) and α-linolenic acid

(C18:3(3)), are obtained exclusively through exogenous sources (Peck, 1994; Garg *et al.*, 1992). C18:2(6) is found in high proportions in the North American diet through the ingestion of corn, safflower and sunflower oils (Peck, 1994; Konturek, 1988). Long chain ω3 fatty acids, eicosapentaenoic acid (C20:5(3)) and docosahexaenoic acid (C22:6(3)), are found primarily in oils of marine origin such as herring, mackerel, and salmon. These fatty acids can also be synthesised *in vivo* from C18:3(3). Food sources of C18:3(3) include linseed oil, soybean oil, canola oil, walnuts, and some dark green leafy vegetables (Lambert-Lagace and Laflamme, 1995).

Membrane Composition and Function

Lipids and proteins are the major constituents of all biological membranes (Clandinin *et al.*, 1985). Phosphoglycerides arranged in a bilayer, two molecules thick, cover the entire surface of individual cells (Wade, 1987; Stubbs and Smith, 1984). This thin film of lipids forms the structural framework of all membranes (Peck, 1994).

The fluid mosaic model, proposed by Singer and Nicholson (1972), forms the bases of current understanding regarding membrane structure. This model depicts a randomly ordered phospholipid bilayer containing mobile proteins, which are embedded to varying degrees in the membrane (Peck, 1994). Recognition of lateral domains within bilayers and subcellular membrane bilayers have changed the original thinking of random organization. It is now thought that domains are heterogenoeus and differentiated, where lipid-lipid and lipid-protein interactions may

be highly specific and influential on membrane structure and function (Peck, 1994; Hargreaves, 1987; Clandinin *et al.*, 1985). Bilayer composition influences the functions of many integral proteins (Thompson, 1992) and its hydrophobic interior acts as a barrier, restricting the flow of dissolved substances (Clandinin *et al.*, 1989; Wade, 1987; Stubbs and Smith, 1984).

Regions of varying fluidity exist within individual membrane bilayers. Therefore, a membrane may contain domains of both liquid-crystalline (fluid) and gel phases (Thompson, 1992). Phospholipid molecules are able to move within or through a bilayer, to form these domains, by three mechanisms: rotational, lateral, and transverse diffusion (Peck, 1994; Cribier *et al.*, 1993; Rhoades and Pflanzer, 1992; Thompson, 1992; Hagve, 1988; Clandinin *et al.*, 1985). Rotational diffusion allows the phospholipid molecule to rotate on its axis. The rate of movement is influenced by the length and degree of unsaturation of the hydrocarbon tails (Cribier *et al.*, 1993; Rhoades and Pflanzer, 1992). Lateral diffusion is very rapid, allowing phospholipids to move along one layer of the plasma membrane (Peck, 1994; Cribier *et al.*, 1993; Rhoades and Pflanzer, 1992; Thompson, 1992). Transverse or 'flip-flop' diffusion requires that the polar head groups of phospholipids from one side of the bilayer move through the non-polar interior of the bilayer to immerge on the other side (Peck, 1994; Cribier *et al.*, 1993; Rhoades and Pflanzer, 1992; Thompson, 1992). This slow process may be a contributing factor to bilayer asymmetry (Rhoades and Pflanzer, 1992; Clandinin *et al.*, 1985). If 'flip-flop' movement were rapid, phospholipids would probably mix randomly and become

more equally distributed between the sides of the bilayer (Rhoades and Pflanzer, 1992).

Dietary fatty acid manipulation alters the composition and physical properties of membranes, and influences the functions of proteins in the membrane (Keelan *et al.*, 1994; Field *et al.*, 1990; Clandinin *et al.*, 1976; Thomson *et al.*, 1989, 1987a). For example, the structure and function of lymphocyte membrane, liver plasma membrane, brain synaptosomes, and cardiac mitochondria are influenced by dietary fat (Clandinin *et al.*, 1985). Changes in the lipid composition of small intestine cell membranes result in alterations in membrane bound protein activities such as enzymes and carrier mediated and passive nutrient transport systems (Keelan *et al.*, 1994,1986; Thomson *et al.*, 1993, 1989, 1988a, 1998b, 1987b, 1986; Rajotte *et al.*, 1987; Thomson and Rajotte, 1983a, 1983b).

The brush border membrane from jejunum and ileum of suckling rats (d 14-20), is much more fluid in nature than brush border membrane of jejunum and ileum of mature rats (d 28-49) (Brasitus and Dudeja, 1988). During maturation, a decrease in membrane fluidity occurs (Hübner *et al.*, 1988). Increases in the saturated to unsaturated fatty acid (w/w), protein to lipid (w/w), and cholesterol to phospholipid (mol/mol) ratios contribute to this decrease in brush border membrane fluidity (Wang *et al.*, 1994; Meddings and Theisen, 1989; Brasitus and Dudeja, 1988; Chu and Walker, 1988; Hubner *et al.*, 1988).

It is accepted that diet fat composition alters the activity of specific membrane bound enzymes. Evidence to suggest dietary fat may influence the

activities of brush border membrane diasaccharidases is limited and controversial. The quantity of fat in diets fed to rats, appears to influence sucrase activity. A decline in sucrase activity is observed with diets containing approximately 73% of energy from fat (Yasutake *et al.*, 1995). It is debatable as to whether this effect is due to a possible influence of fat on membrane function, or that a low carbohydrate level in a high fat diet may be the true factor influencing this decline (Takase and Goda, 1990). Bile acid production is increased in response to a diet high in fat. Higher concentrations of bile acids in the small intestine cause denudation of epithelial cells from intestinal villi, thus increasing degradation of sucrase attached to the mucosal microvilli membrane (Wang *et al.*, 1996; Takase and Goda, 1990). Other studies have demonstrated that disaccharidase activity in rodents is unaffected by diets containing 30% to 40% of energy as fat, even though changes in the structure and function of intestinal enterocyte membranes are observed (Brasitus *et al.*, 1988; Deschryver-Kecskemeti *et al.*, 1991; Dudley *et al.*, 1996; Thomson *et al.*, 1986).

The quality of fat fed has been shown to influence enzyme activity. Takase and Goda (1990) fed rats diets containing long chain triglycerides: corn oil or lard (15% w/w). A reduction in jejunal brush border membrane sucrase activity was observed for both diets when compared to chow fed and MCT (15% w/w) fed rats. Sucrase levels from rats fed the MCT diet were close to control chow fed rats. This evidence suggests that the sucrase activity among these groups was directly related to the type of dietary fat (Goda and Takase, 1994; Takase and Goda, 1990).

Wang and colleagues (1996), fed 20 day old rats semi-purified diets containing either olive oil, MCT, or tallow as the fat source (20% w/w). Sucrase activity was higher in the MCT fed rats as compared to the olive oil and tallow fed rats after 40 hours of feeding. Lactase did not demonstrate this trend. The changes in sucrase activity were transient, as no difference in sucrase activity was observed between groups following 33 days of feeding (Wang *et al.*, 1996). Thus, it was concluded that MCT oil feeding does not have a prolonged effect on hydrolase activity in rat small intestine (Wang *et al.*, 1996).

Dudley and coworkers (1996), examined the effect of feeding pigs diets high in saturated (tallow) verses unsaturated (corn oil) fatty acids on brush border membrane hydrolase activity. Total fat was 15% w/w. Both lactase and sucrase enzyme activities were higher in pigs receiving the high saturated fat diet compared to the high unsaturated fat diet. The amount of C18:0 present in the brush border membrane was highly correlated with both lactase and sucrase activity (Dudley *et al.*, 1996).

The effect of varying fatty acids on disaccharidase activity remains questionable. Further studies utilizing similar quantities of fat with modifications in fatty acid type are required to obtain conclusive evidence pertaining to a role for diet fat on hydrolase activity.

6. SUMMARY

Small intestine development, both pre and postnatal, is dependent upon a variety of growth factors and regulators. Under normal conditions, synthesis of these growth factors occurs within the fetus or within its amniotic environment. Prenatally, maternal growth factors play an indirect role in fetal development. They aid in both the physical and functional development of the placenta, indirectly influencing fetal development through increased nutrient transfer. Postnatally, growth factors are transferred to the neonate through the ingestion of breast milk. Increased levels in colostrum, as opposed to mature milk, are believed to aid the neonatal intestine during this transitional period.

Manipulation of the levels of growth factors delivered to or produced by the fetus are of considerable interest. Possibly, a change in dietary intake of the pregnant or lactating mother may influence these levels *in vivo*. Fatty acid composition of breast milk in rodents can be manipulated by dietary intervention. A possible link between these changes, growth factor concentrations and precocious small intestinal development may exist. Future research will provide insight into this theory.

Chapter 2.

1. RATIONALE

Insulin-like growth factor-1 may play a pivotal role in the development of the gastrointestinal tract. Insulin-like growth factor-1 has been isolated from maternal milk. Its concentration is higher in colostrum than mature milk (Zumkeller *et al.*, 1992; Donovan *et al.*, 1991). Insulin-like growth factor-1 supplementation may be beneficial in accelerating the development of the immature gastrointestinal tract (Zumkeller et al., 1992). Thus the goal of this research is to examine the effect of dietary variations of omega-3 and omega-6 fatty acids and the oral administration of IGF-I on small intestinal maturation using the rat model.

During the first 3 weeks of life, the rat small intestine undergoes rapid growth and development. Studies in the rat have shown that major alterations in brush border membrane enzyme activity occur during the second and third postnatal weeks in preparation for ingestion of solid food (Nsi-Emvo et al., 1996). Normal adult levels are usually observed by day 24-30 of life. Lactase and sucrase have been chosen as they are indicators of functional intestinal maturation. A decline in lactase activity and appearance of sucrase activity is associated with increased cell proliferation and intestinal maturation. Sucrase activity appears and surges at the time that lactase activity declines during the third postnatal week (Olanrewaju *et al.*, 1996; Marti and Fernandez-Otero, 1994; Wild *et al.*, 1993; Liu *et al.*, 1992; Kretchmer, 1985). The time period for these changes is usually between the 15th and 23rd postnatal days. Earlier changes in these disaccharidase activities as a

result of diet, oral IGF-I administration or a combination of both, will be indicative of premature maturation of the small intestine.

The present study examines the effects of maternal dietary fat modification upon the brush border membrane composition of suckling rat pups. Inclusion of C18:3(3), C20:4(6), and C22:6(3) in the diets of nursing dams have been shown to alter the lipid composition of membranes. Possible effects of dietary fatty acids on disaccharidase enzyme activities will be examined. MCT has been shown to increase sucrase and lactase activities while unsaturated long-chain triglycerides have decreased sucrase and lactase enzyme activities in some studies. Thus, we wish to examine the effects of dietary fat modification on suckling rat small intestine lipid composition and disaccharidase enzyme activities.

2. Hypotheses

It is hypothesised that oral IGF-I, in a physiological dose, will result in:

1) a premature appearance of sucrase in the brush border membrane; and

2) a corresponding decline in lactase activity.

Alterations in dietary fat composition of the lactating dams diet will be reflected in brush border membrane fatty acid composition changes in the suckling pup. More specifically:

3) the diet providing and ω6/ω3 ratio of 4:1 will decrease the amount of C20:4(6) in the brush border membrane phospholipid

4) the diet containing 1% AA and 0.7% DHA will increase both C20:4(6) and

C22:6(3) in intestinal phospholipid

5) addition of MCT will result in higher membrane levels of C20:4(6) and C22:6(3), regardless of diet treatment with DHA and/or AA.

3. Objectives

These hypotheses will be tested by: 1) administering IGF-I orally to suckling pups on days 10 through 13 of life and 2) feeding lactating dams nutritionally complete diets varying in fat composition.

Chapter 3.

MATERIALS AND METHODS

1. Animals and Diets

Eight confirmed pregnant Sprague-Dawley rats were obtained from Health Sciences Laboratory Animal Services. Animals were housed individually in plastic cages. Room conditions were kept at 21 °C with 12 hour periods of light and darkness. Water and food was supplied *ad libitum*. The day of birth was designated as day zero. Two dams were assigned to one of four experimental diets on day one. Litters were culled to ten pups on day two of life. On postnatal day ten, each litter was divided into two groups of five. Pups (n=5) in the first group received an oral gavage (46 ng IGF-I in 50 µl phosphate buffered saline) of insulin-like growth factor-1 (IGF-I) (Sigma Biosciences, St Louis, MO, USA). The remaining pups (n=5) received an oral gavage, 50 µl, of phosphate buffered saline (PBS). IGF-I and PBS gavages were administered daily for four consecutive days beginning on day ten of life through day thirteen. All litters were sacrificed on the morning of postnatal day fourteen.

Semi-purified diets containing 20% (w/w) fat were used. Composition of the basal diet is provided **(Table 3.1)**. The basal fat blend, containing an ω6/ω3 fatty acid ratio of 7.3:1, was formulated to contain the fat composition present in SMA® an infant formula marketed by Wyeth Ayerst (Randor, Pennsylvania, USA). This fat blend was achieved by blending 21.4% canola oil, 24.4% coconut oil, 19.1% corn

oil, and 35.1% oleo oil. Four experimental diets were formulated by addition of various combinations of docosahexaenoic acid (DHA), arachidonic acid (AA), and/or medium chain triglycerides (MCT) to the basal fat blend. Diet fat composition was determined by gas chromatography **(Table 3.2)**. DHA and AA triglycerides were obtained from single cell oils (Martek Biosciences Corp., Colombia, Maryland, USA). MCT oil was purchased from Wyeth-Ayerst, Ville St. Laurent, Quebec.

Table 3.1
Basal Diet Composition per Kilogram of Diet

Ingredient	Amount (g)
Casein	270
Starch	200
Glucose	207
Non-Nutritive Fibre	50
Vitamin Mix[1]	10
Mineral Mix[2]	51
Choline chloride	3
Inositol	6
L-Methionine	3
$MnSo_4 \cdot H_20$[3]	0.2
$Na_2Se0_3 \cdot 5H_20$	0.2
Fat Mix[4]	200
Total Diet Weight	1000

1 AOAC Vitamin Mix (Teklab Test Diets, Madison, WI.,USA)
2 Bernhardt-Tomarelli Mineral Mix (General Biochemicals, Chargrin Fall, OH.,USA)
3 $MnSo_4 \cdot H_20$ is added at 0.0445% in dextrose
4 Fatty-acid composition as shown in Table 3.2

Table 3.2 Fatty Acid Composition of Diets Fed

Fatty Acid (% w/w)	DHA+AA	ω6/ω3 4:1	MCT + DHA + AA	MCT + DHA
C8:0	0	1.2	30.0	30.0
C10:0	1.4	1.3	15.1	14.5
C12:0	11.8	11.2	6.8	7.1
C14:0	6.4	5.9	3.7	3.9
C14:1(7)	0.1	0.1	0.0	0.0
C16:0	14.2	13.4	8.1	8.2
C16:1(7)	0.1	0.1	0.0	0.0
C18:0	6.6	6.5	3.7	3.7
C18:1(7+9)	36.5	36.0	20.0	20.7
C18:2(6)	16.0	16.5	8.6	9.0
C18:3(3)	2.2	4.6	1.1	1.2
C20:0	0.3	0.2	0.1	0.1
C20:1(9)	0.5	0.5	0.1	0.2
C20:4(6)	0.9	0.1	1.1	0.0
C22:6(3)	0.6	0.1	0.7	0.7

2. Insulin-Like Growth Factor-1 Reconstitution

IGF-I (Sigma Biosciences, St. Louis, MO, USA) was dissolved in 10 mM HCl to obtain a concentration of 0.5 mg/ml. An aliquot of the reconstituted insulin-like growth factor-1 was added to phosphate buffered saline (0.14 M NaCl, 0.003 M KCl, 0.008 M Na_2HPO_4, 0.001 M KH_2PO_4, pH 7.4) with bovine serum albumin (0.1% g/v) to obtain a concentration of 46 ng/50 µl.

3. Removal of Small Intestine

Animals were sacrificed on postnatal day 14. Cardiac punctures were performed, under halothane, to obtain a 1.0-1.5 ml blood sample. Following cervical dislocation the small intestine was removed from the ligament of Treitz to the ileocecal valve. Intestinal contents were removed by flushing the small intestine with ice cold 0.89% saline. The intestine was weighed and divided into three equal portions designated ileum, middle, and jejunum. Each portion was cut longitudinally and a prechilled glass slide was used to scrape off the mucosa. Scrapings were stored at -70 °C prior to membrane isolation and enzymatic analysis (Malathi *et al.*, 1979; Schmitz *et al.*, 1973). Scraping samples were randomized prior to brush border membrane isolation.

4. Brush Border Membrane Isolation

Jejunal and ileal brush border membranes were isolated using sequential techniques of $CaCl_2$ precipitation, differential centrifugation, and sonication (Malathi *et al.*, 1979; Schmitz *et al* .,1973).

5. Sucrase and Lactase Assays

Intestinal disaccharidase activity was assayed using a Gluco-quant Glucose Reagent Kit (Sigma St. Louis, Missouri, USA) (Dahlqvist, A., 1964). Purity of the membrane preparation was estimated by enrichment of lactase activity (Koldovsky and Dahlqvist, 1969). A Unit (U) of activity is defined as µmols disaccharide hydrolysed per minute at 37 °C.

6. Protein Determination

Protein concentrations were determined using the Bio-Rad Protein Assay Kit based on the method of Bradford (1976).

7. Lipid Analysis

Lipids were extracted from BBM pellets using a modified Folch procedure (Folch *et al.*, 1957). Each BBM pellet (25 µl) was suspended in 1.0 ml chloroform:methanol 2:1 (v/v) and 0.05% (v/v) ethoxyquine. $CaCl_2$ (0.25 ml) was added to each tube. Tubes were vortexed and then centrifuged (5 minutes at 2000 rpm). Upon phase separation, the upper methanol layer was discarded. The lower chloroform layer was transferred to a clean tube and dried under nitrogen. The dried samples were then reconstitiuted with 100 µl chloroform and stored at -70 °C.

A. Thin-Layer Chromatography

Separation of individual phospholipid was performed on Analtech Silica Gel H-plates (10 x 10 cm, Analtech, Newark, DE, USA). Plates were heat activated for 1 hour at 110 °C. Samples were spotted on activated H-plates. Phospholipid standards (Sigma), phosphatidylcholine (PC), phosphatidylinositol (PI),

phosphatidylserine (PS), and phosphatidylethanolamine (PE), were spotted on each H-plate. Plates were developed in tanks lined with Watman #1 filter paper containing the following solvent system: chloroform:methanol:2-propanol:triethylamine:0.25% KCl (30:9:25:18:6 by volume) for approximately 55 minutes (Touchstone *et al.*, 1980). Plates were air dried at room temperature and visualized with 0.1% (w/v) 8-anilino-1-napthalene-sulfonic acid (ANSA) in water. Individual phospholipid bands, PC, PI, PS, and PE, were identified and scraped into screw cap test tubes. For quantification, 1 µg heptadecanoic acid (Sigma, Mississauga, Ontario, Canada) was added to each tube as an external standard.

B. Methylation of Fatty Acids

Fatty acid methyl esters were prepared using BF_3/methanol reagent (Morrison and Smith, 1964). To each tube containing individual phospholipid bands, 1.0 ml 14% (w/v) BF_3/methanol and 2 ml hexane were added (Morrison and Smith, 1964). Samples were placed in a 100-110 °C sandbath for 1 hour, cooled to room temperature, and separated into the hexane phase following addition of 1 ml water. The hexane phase, containing the methyl esters, was removed, dried under nitrogen, and stored in sealed microvials at -70 °C.

C. Gas-Liquid Chromatography

Fatty acid methyl ester analysis was performed with the Vista 6000 GLC and Star Chromatography Workstation Version 4.0 data system (Varian, Georgetown, Ontario, Canada), using a fused silica BP20 capillary column (25m x 0.25mm I.D.; Scientific Glass Engineering PTY. LTD., Ringwood, Victoria, Australia). A carrier

gas, helium, was used at a flow rate of 1.5 ml/minute with the inlet splitter set at 100:1. The initial oven temperature of 90 °C was increased to 172 °C in increments of 20 °C/minute and held for 14.5 minutes followed by an increase to 220 °C by 2.5 °C/minute and then held for 13 minutes. Injection and detection temperature was 250 °C. Fatty acid methyl esters were identified by comparison of retention data with that of authentic standards (Sigma, Mississauga, Ontario, Canada; Supelco, Mississauga, Ontario, Canada), and quantitated by peak area comparison with the heptadecanoic acid internal standard (Sigma, Mississauga, Ontario, Canada).

8. Statistical Analysis

Results are expressed as means ± standard error of the mean. The effect(s) of diet treatment, litter, IGF-I treatment, gender, and intestinal section were compared using a nested-factorial analysis of variance procedure (Harvey, 1975) using SAS (Version 6.04, SAS Institute, Cary, NC, USA). Significant effects ($p<0.05$) were further defined; Individual treatment effects were determined by using the Duncan's multiple range test (Steel and Torrie, 1980). Interactions between variables were analysed using a p-diff test (Steel and Torrie, 1980). Significant differences were not observed between male and female pups for animal characteristics and enzyme assays. Thus, the statistical analysis combines both males and females for analysis of the effect of diet and IGF-I treatment on these variables.

The effect(s) of diet, IGF-I treatments, and intestinal section on the fatty acid composition of small intestine phospholipid was determined by least-squared three-way analysis of variance procedures (Harvey, 1975). A Duncan's multiple range

test was used to discriminate significant differences ($P<0.05$) due to diet treatment, IGF-I treatment, and intestinal section (Steel and Torrie, 1980). Interactions between diet, treatment, and intestinal section were analysed using a p-diff test (Steel and Torrie, 1980). Correlations between lactase activity and individual fatty acid percentages were determine by Simple Regression Analysis using Excel.

Chapter 4.

RESULTS

1. Animal Characteristics

A. Gender

Gender had no effect on pup body weight, intestinal weight, intestinal length, or brush border membrane activities.

B. Body Weight

Mean body weights for control and IGF-I treated pups were obtained on days 10 through 13 for each diet treatment. IGF-I treatment had no effect on body weight during this period. Rat pups from IGF-I and control treated groups were pooled for body weight data. Pups from dams fed the MCT+DHA diet exhibited lower body weights during this period than the ω6/ω3 4:1 diet treatment group **(Table 4.1)**. Body weight gain over this period was also lower for these pups **(Table 4.1)**. Within each respective diet treatment group, body weights increased over the three day period. These daily increases were significant. Individual animals were weighed on the morning of sacrifice. Dam diet treatment had no effect on pup final body weight **(Table 4.1)**. Final body weights were not different between control and IGF-I treated pups.

C. Intestinal Weight

Pup intestinal weight (g) was expressed as a percent of body weight (g). Diet treatment of dams had no effect on pup intestinal weight. Pups receiving IGF-I had lower intestinal weights when compared to controls **(Figure 4.1)**. Intestinal weight

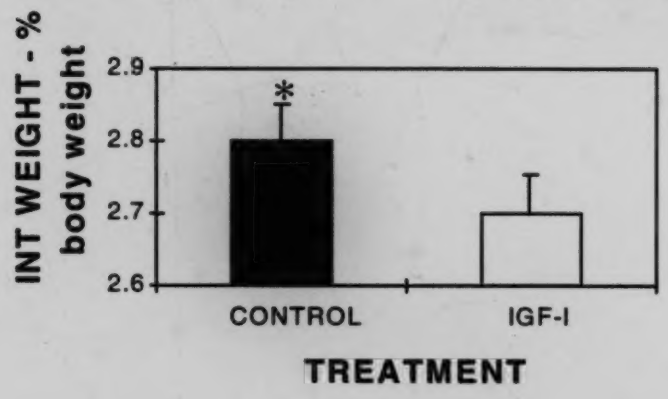
(g) per centimetre of intestine was not different regardless of treatment.

Table 4.1
Effect of Diet on Pup Body Weight

Diet	Final Body Weight g	Mean Weight g/3 days	Mean Weight Gain g/3 days
DHA+AA	34.3±1.1	32.0±1.4^{ab}	2.8±0.2^{x}
ω6/ω3 4:1	37.7±1.1	33.5±1.2^{a}	3.0±0.2^{x}
MCT+DHA+AA	34.1±1.1	32.2±1.2^{ab}	2.7±0.2^{xy}
MCT+DHA	33.1±1.1	28.6±1.2^{b}	2.2±0.2^{y}

Values are means± SEM (n=10 for final body weight; n=10 for mean body weight and mean weight gain). Comparisons between diet treatments are significantly different at: ^{a,b} $p< 0.04$; ^{x,y} $p< 0.03$.

Figure 4.1
Effect of IGF-I Treatment on Intestinal Weight (% Body Weight)

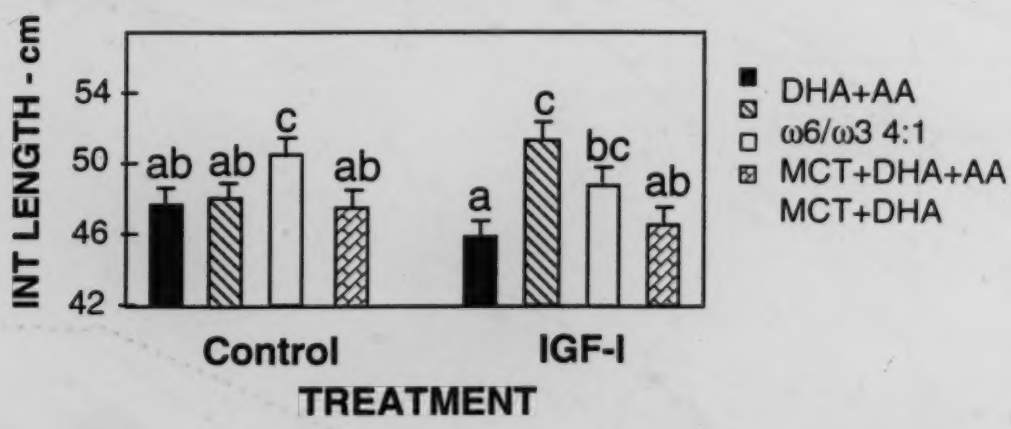


Values are means±SEM (n=40). * P<0.02

D. Intestinal Length

When compared to control littermates, intestinal length (cm) was greater in IGF-I treated pups from dams fed the diet providing an ω6/ω3 ratio of 4:1 **(Figure 4.2)**. Intestinal length of IGF-I treated pups from the DHA+AA diet treatment were shorter than IGF-I treated pups from both the ω6/ω3 4:1 and MCT+DHA+AA diet treatment groups **(Figure 4.2)**. Control pups from dams fed the MCT+DHA+AA diet displayed a greater intestinal length than the remaining control pups **(Figure 4.2)**.

Figure 4.2
Effect of Diet and IGF-I Treatment on Intestinal Length

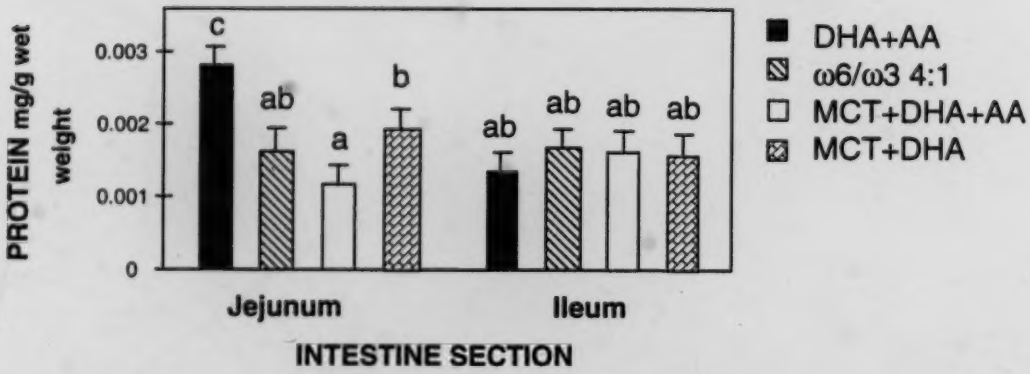


Values are means±SEM (n=10). Comparisons with unlike superscripts are significantly different at P<0.02.

2. Brush Border Membrane Protein Concentration

IGF-I had no effect on brush border membrane protein concentration, regardless of diet treatment. Jejunal protein concentrations of pups from dams fed the DHA+AA diet displayed the highest concentration of membrane protein **(Figure 4.3)**. Differences between intestinal sections were not observed for the ω6/ω3 4:1, MCT+DHA+AA, and MCT+DHA diet treatment groups **(Figure 4.3)**.

Figure 4.3
Effect of Diet on Brush Border Membrane Protein Concentration



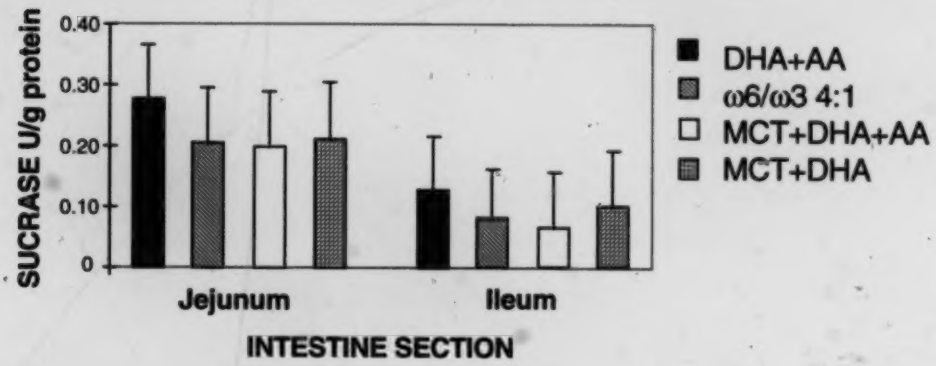
Values are means±SEM (n=20). Comparisons with unlike superscripts are significantly different at P<0.0003.

3. Sucrase Activity

When sucrase activity was expressed as units per gram of protein **(Figure 4.4)**, intestinal weight, mucosal weight, or body weight, activities did not differ for

any of the parameters examined.

Figure 4.4
Effect of Diet on Brush Border Membrane Sucrase Activity



Values are means± SEM (n=20).

4. Lactase Activity

The purity of brush border membrane isolates were on the range of 10- to 15-fold based on the specific activity of lactase.

A. Units per Gram of Protein

Diet and IGF-I treatment, alone or in combination, had no effect on lactase activity when expressed as units per gram of protein. Differences between intestinal regions, jejunum and ileum, were not observed **(Table 4.2)**.

B. Units per Gram of Mucosal Weight

IGF-I treatment had no effect on lactase activity when expressed as units per gram of mucosal weight. Diet effects were observed between intestinal sections. Jejunal lactase activity was the highest in pups from dams fed the DHA+AA diet **(Table 4.2)**. The remaining diet treatments did not exhibit differences between jejunal and ileal sections **(Table 4.2)**.

C. Units per Gram of Intestinal Weight

Diet and IGF-I treatment alone had no effect in lactase activity when expressed as units per gram of intestine. Differences were observed between intestinal sections where the jejunum displayed higher lactase activity than the ileum. Higher jejunal lactase activity when compared to ileal activity was observed in pups from dams fed the DHA+AA and MCT+DHA diets **(Table 4.2)**.

D. Units per Gram of Body Weight

Lactase activity was not affected by IGF-I treatment when expressed as units per gram of body weight. Jejunal lactase activity was higher than ileal lactase activity in pups from dams fed the DHA+AA and MCT+DHA diets **(Table 4.2)**. Jejunal values were higher in these groups when compared to the ω6/ω3 4:1 and MCT+DHA+AA groups **(Table 4.2)**. Ileal values were not different among diet treatments **(Table 4.2)**.

Table 4.2
Effect of Diet on Jejunal and Ileal Brush Border Membrane Lactase Activity

	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
Units / g Protein				
Jejunum	61±10	57±9	110±9	70±9
Ileum	53±9	64±9	75±9	70±10
Units / g mucosa				
Jejunum	1.31±0.14^{c}	0.57±0.13^{ab}	0.74±0.13^{ab}	0.71±0.13^{ab}
Ileum	0.43±0.13^{a}	0.65±0.13^{ab}	0.89±0.13^{b}	0.91±0.13^{b}
Units / g intestine				
Jejunum	0.18±0.01^{z}	0.08±0.01^{x}	0.10±0.01^{xy}	0.12±0.01^{y}
Ileum	0.06±0.01^{x}	0.07±0.01^{x}	0.10±0.01^{xy}	0.08±0.01^{x}
Units / g body weight (x 10^{-4})				
Jejunum	42 ±3 *	25±3	26±3	37±3 *
Ileum	21±3	22±3	24 ±3	22±3

Values are means± SEM (n=20). Significant differences between diet treatments and intestinal sections for each expression of lactase activity are indicated as: ^{a,b,c} $p<0.001$; ^{x,y,z} $p<0.0003$; * $p<0.002$. U = µmol lactose hydrolysed per minute at 37°C.

5. Brush Border Membrane Phospholipid Composition

A. Phosphatidylcholine

An effect of diet was observed for C18:1(7+9). Pups from dams fed the MCT+DHA+AA diet exhibited lower levels of C18:1(7+9) **(Table 4.3)**. Regional intestinal differences were observed for membrane phosphatidylcholine content of C16:0, C18:2(6), and C20:4(6) **(Table 4.4)**. Higher levels of C16:0 and C20:4(6) were observed in the ileum **(Table 4.4)**. Higher ileal C20:4(6) content was observed when compared to jejunal C20:4(6) levels in pups from dams fed the ω6/ω3 4:1,

MCT+DHA+AA, and MCT+DHA diets **(Table 4.5)** C18:2(6) was higher in the jejunum than in the ileum **(Table 4.4)**. Jejunal C22:6(3) content of pups from dams fed the MCT+DHA+AA diet was higher than in the corresponding ileal segment **(Table 4.5)**.

Ileal brush border membrane phosphatidylcholine C20:4(6) composition was affected by IGF-I treatment. Ileal C20:4(6) levels were higher than jejunal levels in control pups **(Table 4.13)**. The C20:4(6) content of ileum in control pups was higher than that of the IGF-I treated pups **(Table 4.13)**.

Table 4.3
Effect of Diet on the Fatty Acid Composition of Brush Border Membrane Phosphatidylcholine (%w/w)

Fatty Acid	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
C16:0	24.5±0.9	24.3±0.9	26.2±0.9	27.1±0.9
C18:0	27.4±2.2	28.7±2.1	28.3±2.1	25.4±2.1
C18:1(7+9)	8.4±1.3	8.1±1.2	3.9±1.2*	7.8±1.2
C18:2(6)	12.9±1.9	16.2±1.8	9.7±1.8	13.4±1.8
C18:3(3)	0.1±0.4	0.9±0.4	0.2±0.4	0.3±0.4
C20:4(6)	10.0±1.0	8.2±0.9	9.9±0.9	6.7±0.9
C20:5(3)	0.5±0.3	0.3±0.3	1.1±0.3	1.1±0.3
C22:6(3)	3.3±0.4	2.4±0.4	3.7±0.4	3.1±0.4

Values are means± SEM (n=12). Comparisons between diet treatments within a phospholipid are significantly different at $p<0.05$.

Table 4.4
Fatty Acid composition of Phosphatidylcholine (%w/w) in Jejunum and Ileum

Fatty Acid	Jejunum	Ileum
C16:0	23.0±0.6	28.0±0.6*
C18:2(6)	17.3±1.3*	8.7±1.3
C20:4(6)	5.6±0.6	11.8±0.7*

Values are Means±SEM (n=24). Comparisons between intestinal sections within a fatty acid are significantly different at $p<0.0001$.

Table 4.5
Effect of Diet on the Fatty Acid Composition of Phosphatidylcholine (%w/w) in Jejunum and Ileum

	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
C20:4(6)				
Jejunum	8.3 ± 1.3^{ab}	4.8 ± 1.3^{a}	4.7 ± 1.3^{a}	4.6 ± 1.3^{a}
Ileum	11.7 ± 1.5^{bc}	11.5 ± 1.3^{bc}	15.1 ± 1.3^{c}	8.8 ± 1.3^{b}
C22:6(3)				
Jejunum	4.0 ± 0.6^{yz}	2.5 ± 0.5^{xy}	4.7 ± 0.5^{z}	2.5 ± 0.5^{xy}
Ileum	2.6 ± 0.6^{xy}	2.3 ± 0.5^{x}	2.8 ± 0.5^{xy}	3.7 ± 0.5^{xyz}

Values are Means±SEM (n=6). Comparisons between intestinal sections and diet treatments within a fatty acid are significantly different at: ^{a,b,c} $p<0.05$; ^{x,y,z} $p<0.04$.

B. Phosphatidylethanolamine

Brush border membrane phosphatidylethanolamine levels of C18:2(6) and C22:6(3) were influenced by diet treatment **(Table 4.6)**. The level of C18:2(6) was

highest in pups from dams fed the diet providing an ω6/ω3 ratio of 4:1 **(Table 4.6)**.

Table 4.6
Effect of Diet on the Fatty Acid Composition of Brush Border Membrane Phosphatidylethanolamine (%w/w)

Fatty Acid	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
C16:0	21.7±1.3	21.0±1.3	23.2±1.3	22.6±1.2
C18:0	29.6±1.8	29.0±1.8	29.2±1.8	29.0±1.8
C18:1(7+9)	4.0±1.7	7.7±1.6	4.5±1.6	6.0±1.6
C18:2(6)	5.2±0.5	7.6±0.5**	4.7±0.5	5.6±0.5
C18:3(3)	0.3±0.3	0.8±0.3	0.0±0.3	0.8±0.3
C20:4(6)	8.2±1.2	9.4±1.2	7.1±1.1	5.9±1.1
C20:5(3)	0.5±0.5	0.7±0.5	1.8±0.5	1.3±0.5
C22:6(3)	7.0±1.0^{ab}	4.3±1.0^{b}	6.8±1.0^{ab}	8.6±1.0^{a}

Values are means± SEM (n=12). Comparisons between diet treatments within a phospholipid are significantly different at: ^{a,b} $p<0.05$; ** $p<0.003$.

The level of C22:6(3) was lowest in this group **(Table 4.6)**. Regional differences were observed for C16:0 and C18:2(6) **(Table 4.7)**. Ileal content of C16:0 was higher than jejunal content of C16:0 **(Table 4.7)**. Higher levels of C16:0 were observed in the ileum as compared to the jejunum of pups from dams fed the diets providing DHA+AA and an ω6/ω3 ratio of 4:1 **(Table 4.8)**. Differences in C16:0 content were not observed between the intestinal sections for pups from the MCT+DHA+AA and MCT+DHA treatment groups **(Table 4.8)**. C18:2(6) levels were

higher in jejunum than ileum **(Table 4.7)**.

Table 4.7
Fatty Acid Composition of Phosphatidylethanolamine (%w/w) in Jejunum and Ileum

Fatty Acid	Jejunum	Ileum
C16:0	20.3±0.9	23.9±0.9*
C18:2(6)	7.6±0.4†	3.9±0.4

Values are Means±SEM (n=24). Comparisons between intestinal sections within a fatty acid are significantly different at: * $p<0.01$; † $p<0.0001$.

Table 4.8
Effect of Diet on the Fatty Acid Composition of Phosphatidylethanolamine (%w/w) in Jejunum and Ileum

	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
C16:0				
Jejunum	17.8±1.7^{a}	18.1±1.7^{a}	24.4±1.7^{b}	21.1±1.7^{ab}
Ileum	25.6±2.0^{b}	23.9±2.0^{b}	21.9±1.7^{ab}	24.2±1.7^{b}
C22:6(3)				
Jejunum	9.6±1.4^{yz}	4.4±1.4^{x}	7.8±1.4^{xy}	3.8±1.4^{x}
Ileum	4.4±1.6^{x}	4.3±1.5^{x}	5.9±1.4^{xy}	13.4±1.4^{z}

Values are Means±SEM (n=6). Comparisons between intestinal sections and diet treatments within a fatty acid are significantly different at: ^{a,b,c} $p<0.04$; ^{x,y,z} $p<0.0001$.

Jejunal C22:6(3) levels were higher than ileal values in pups from dams fed the

DHA+AA diet **(Table 4.8)**. Ileal values of C22:6(3) were higher than jejunal values in pups from dams fed the MCT+DHA diet treatment **(Table 4.8)**. A higher C22:6(3) level was observed in control pups from dams fed the MCT+DHA diet **(Table 4.9)**. A difference in ileal C22:6(3) content in control and IGF-I treated pups was observed. Jejunal C22:6(3) levels were not different . The ileal C22:6(3) content in control pups was higher than the ileal content of IGF-I treated pups **(Table 4.13)**.

Table 4.9
Effect of IGF-I and Diet on Phosphatidylethanolamine C22:6(3) (%w/w)

C22:6(3)	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
Control	5.4 ± 1.4^{ab}	4.3 ± 1.4^{a}	7.2 ± 1.4^{ab}	11.6 ± 1.4^{c}
IGF-I	8.5 ± 1.6^{bc}	4.4 ± 1.6^{ab}	6.4 ± 1.4^{ab}	5.6 ± 1.4^{ab}

Values are Means±SEM (n=6). Comparisons with unlike superscripts are significantly different at $p<0.03$.

C. Phosphatidylserine

Diet had an effect on phosphatidylserine C18:2(6) and C20:4(6) levels **(Table 4.10)**. C18:2(6) levels were highest in pups from dams fed the DHA+AA diet **(Table 4.10)**. Levels of C20:4(6) were highest in pups from dams fed the MCT+DHA diet **(Table 4.10)**. This level was not higher than that observed for pups in the DHA+AA diet treatment group. Levels of C18:2(6) were observed to be higher in the jejunum, regardless of diet treatment **(Table 4.11)**. Jejunal C18:2(6) levels were found to be

highest in pups from dams fed the DHA+AA diet **(Table 4.12)**. C20:4(6) was higher in jejunum than ileum **(Table 4.11)**. The highest levels of C20:4(6) were observed in the jejunum of pups from dams fed the DHA+AA and MCT+DHA diets **(Table 4.12)**. Phosphatidylserine C18:0 levels varied between intestinal sections depending upon diet treatments. Ileal C18:0 levels were higher than jejunal levels in pups from dams fed the diet providing an ω6/ω3 ratio of 4:1 and the MCT+DHA diet **(Table 4.12)**.

Table 4.10
Effect of Diet on the Fatty Acid Composition of Brush Border Membrane Phosphatidylserine (%w/w)

Fatty Acid	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
Phosphatidylserine				
C16:0	29.0±2.0	27.1±2.5	31.9±3.5	25.7±2.7
C18:0	23.3±2.6	23.1±2.2	22.6±1.4	26.0±2.3
C18:1(7+9)	9.1±1.9	5.6±1.4	7.4±1.9	5.2±1.5
C18:2(6)	4.3±0.5*	2.1±0.4	2.0±0.6	3.0±0.4
C18:3(3)	0.2±0.3	0.1±0.3	0.5±0.3	0.6±0.3
C20:4(6)	1.5±0.3^{bc}	1.0±0.3^{ab}	1.0±0.2^{a}‡	2.2±0.4^{c}
C20:5(3)	0.8±1.0	0.7±1.0	2.2±1.4	3.0±1.1
C22:6(3)	2.8±1.6	5.3±0.8	4.1±0.9	5.1±0.7

Values are means± SEM (n=12). Comparisons between diet treatments within a phospholipid are significantly different at: *p<0.0004; ^{a,b,c}p<0.006.
‡ data may be unreliable due to the low number of observations (n=2)

Table 4.11
Fatty Acid Composition of Phosphatidylserine (%w/w) in Jejunum and Ileum

Fatty Acid	Jejunum	Ileum
C18:2(6)	4.2±0.8*	1.7±0.3
C20:4(6)	2.6±0.6†	0.4±0.1

Values are Means±SEM (n=24). Comparisons between intestinal sections within a fatty acid are significantly different at: * $p<0.0001$; † $p<0.0001$.

Table 4.12
Effect of Diet on the Fatty Acid Composition of Phosphatidylserine (%w/w) in Jejunum and Ileum

	DHA+AA	ω6/ω3 4:1	MCT+DHA+AA	MCT+DHA
C18:0				
Jejunum	20.8 ± 1.1^{abc}	14.0 ± 3.2^{a}	18.4 ± 5.0^{abc}	33.1 ± 3.5^{d}
Ileum	24.4 ± 3.2^{bcd}	28.6 ± 2.9^{cd}	26.8 ± 3.2^{bcd}	18.9 ± 2.9^{ab}
C18:2(6)				
Jejunum	9.8 ± 0.7^{z}	2.4 ± 0.6^{xy}	2.8 ± 0.9^{xy}	4.3 ± 0.7^{y}
Ileum	2.2 ± 0.6^{x}	1.7 ± 0.5^{x}	1.2 ± 0.6^{x}	1.8 ± 0.5^{x}
C20:4(6)				
Jejunum	4.5±0.04*	1.3±0.5	0.6±0.8	4.0±0.6*
Ileum	0.7±0.5	0.6±0.5	0.1±0.5	0.4±0.5

Values are Means±SEM (n=6). Comparisons between intestinal sections and diet treatments within a fatty acid are significantly different at: ^{a,b,c} $p<0.002$; ^{x,y,z} $p<0.006$; * $p<0.014$.

D. Phosphatidylinositol

Phosphatidylinositol was present in negligible amounts, therefore samples

were not analysed.

E. Effect of IGF-I on Phospholipid Fatty Acid Composition

IGF-I influenced the levels of ileal C20:4(6) and C22:6(3) **(Table 4.13)**. Control pups had higher ileal levels of phosphatidylcholine C20:4(6) and phosphatidylethanolamine C22:6(6) **(Table 4.13)**. No effect was observed for the jejunum.

Table 4.13
Effect of IGF-I on Phospholipid Fatty Acid Composition (%w/w)

	Control	IGF-I
Phosphatidylcholine C20:4(6)		
Jejunum	5.3 ± 0.9^{a}	6.0 ± 0.9^{ab}
Ileum	13.5 ± 0.9^{c}	10.1 ± 1.0^{b}
Phosphatidylethanolamine C22:6(3)		
Jejunum	5.7 ± 1.0^{xy}	7.1 ± 1.0^{xy}
Ileum	8.5 ± 1.0^{x}	5.4 ± 1.1^{y}

Values are means± SEM (n=12). Comparisons with unlike superscripts are significantly different at: ^{a,b,c} $P<0.034$; ^{x,y} $P<0.034$.

F. Relationship Between Lactase Activity and Phospholipid Fatty Acids

Levels of phosphatidylcholine and phosphatidylethanolamine C20:4(6) correlated negatively with membrane lactase activity, when expressed as units per gram of protein. Lactase activity increased with rising levels of phosphatidylcholine

C22:6(3). Correlations were not observed between lactase activity and phosphatidylserine fatty acid composition.

Chapter 5

DISCUSSION

Animal Characteristics

Weight of the small intestine (% body weight) was found to be lower in IGF-I treated pups. Previous literature does not offer an explanation for the lower intestinal weights (% body weight) observed in these rat pups, as this variable has been reported to remain unchanged or to increase following moderate to high oral treatment with IGF-I (Young *et al.*, 1990; Xu, *et al.*, 1994). Oral administration of IGF-I (1 µg/day) has been reported to have no effect on suckling rat pup body or intestinal weight when administered on days 10 through 15 of life (Young *et al.*, 1990). Infusion of IGF-I has been found to increase total body weight during periods of increasing GH dependency, such as late weaning (Steeb *et al.*, 1997). When IGF-I was administered subcutaneously to normal adult rats, at a dose of 278 µg/day, body weight and intestinal weight (% body weight) did not increase after three days (Steeb *et al.*, 1994). A dose-dependent increase in both body weight and intestinal weight (% body weight) was observed following 14 days of subcutaneous IGF-I treatment at doses of 111 and 278 µg/day (Steeb *et al.*, 1995). The dosage presented in these studies was much higher (>1000 fold) than the physiological dose used in this experiment (46 ng/day). A possible explanation is as follows: intestinal lengths of IGF-I treated pups were shorter (not significantly) when compared to littermate controls, with the exception of the ω6/ω3 4:1 diet group. Intestinal weight as a percentage of intestinal length and final body weight

was not different between IGF-I treated and control pups. Thus, the somewhat shorter length of the IGF-I pup intestine would suggest a lower intestinal mass which may have attributed to the significant difference observed when intestinal weight was expressed as a percentage of body weight.

Protein Concentrations

IGF-I treatment had no effect on brush border membrane protein concentrations. In previous studies, protein concentrations were not influenced by oral IGF-I administration to suckling rats (Young *et al.*, 1990) and suckling pigs (Xu *et al.*, 1994) nor by intra luminal infusion of IGF-I in normal adult rats (Steeb *et al.*, 1994). Intestinal sections displayed differences in the DHA+AA diet treatment group. The jejunal segment contained a significantly higher concentration of protein when compared to the ileum. A previous study, utilizing diets similar to those in the present experiment, did not observe changes in small intestine protein concentrations (Thomson *et al.*, 1986). Thus, the cause of the increased protein concentration in response to dietary treatment is unknown.

Enzyme Activity

An objective of this research was to examine a possible effect of IGF-I on small intestine disaccharidase activities. Stimulation of jejunal lactase activity by oral IGF-I administration to suckling rat pups has been previously reported (Young *et al.*, 1990). Exogenous hormones, such as corticosterone and thyroxine,

administered by various means during the second postnatal week to rodents have been shown to induce the precocious appearance of sucrase activities accompanied by an early decline in lactase activity (Nsi-Envo *et al.*, 1996; Shinder *et al.*, 1995; Hodin *et al.*, 1994; Menard and Calvert, 1991; Yeh *et al.*, 1991b; Sheard and Walker, 1988). Exogenous IGF-I treatment in the present study had no effect on observed lactase and sucrase activities. The physiological dose given may have been below the required levels to elicit a response or the treatment period may have been to short. Previous studies have demonstrated that the disaccharidase activity patterns of suckling rat pup small intestine are capable of change. It would be of interest to examine if disaccharidase enzyme activities respond to a physiological dose of IGF-I when treatment begins at birth and continues to set time intervals up to two weeks of age.

Jejunal lactase activity, expressed as units per gram of mucosal weight, was higher in pups from dams fed the DHA+AA diet. An increase in jejunal lactase activity was not observed in the MCT+DHA+AA diet treatment group. This observation suggests that the presence of MCT may have influenced this dietary response. Without examination of milk MCT levels, a definitive answer can not be reached. Ileal lactase activity was significantly higher in the MCT diet treatment groups when compared to the ileal levels from the DHA+AA diet treatment group. As lactase is predominately found in the brush border membrane of suckling and adult jejunum (Koldovsky, 1981), it is surprising that ileal levels were not lower than jejunal levels for pups from dams fed either MCT+DHA+AA, MCT+DHA, or the diet

providing an ω6/ ω3 ratio of 4:1.

When examining the lactase activity as a function of intestine and body weight, jejunal lactase activity was reported to be highest in pups from dams fed the DHA+AA diet. Prior to weaning Increased lactase activity may be beneficial to suckling animals, as their sole source of nutrition is maternal milk containing high concentrations of lactose. A decline in lactase prior to weaning would be detrimental to an animal not yet consuming solid food in their diet. When lactase levels are sufficiently low, lactose remains unhydrolyzed in the intestinal lumen (Thomson *et al.*, 1994). By its osmotic nature, lactose facilitates fluid accumulation in the lumen which leads to osmotic diarrhea. Fluid, electrolyte and nutrient losses result (Thompson *et al.*, 1994).

Sucrase activity was not influenced by diet or IGF-I treatment in the present study. However, sucrase activity was detected. Kaouass and colleagues (1996) and Wèry and Dandrifosse (1993) have demonstrated that sucrase activity is undetectable in 14 and 11 day old suckling rats, respectively. It remains absent for the first two weeks of postnatal life and begins to surge following the 15th day (Wild *et al.*, 1993; Menard and Calvert, 1991; Kretchmer, 1985; Koldovsky, 1981). Our present findings suggest that sucrase activity, albeit low, is expressed in brush border membranes as early as 14 days of life prior to the intake of solid food.

A correlation between sucrase and lactase specific activity was not observed. A surge in sucrase activity, which normally occurs between days 15 and 18 of life, was not observed in this study. Thus, the hypothesis that lactase levels would

decline in response to precociously increased sucrase levels, as a result of diet and IGF-I treatment, could not be determined from this experiment.

Brush Border Membrane Fatty Acid Composition

Gender was not included in the analysis of brush border membrane phospholipid composition as it was not found to be significant for animal characteristics and enzyme activities. Male and female suckling rat pups are commonly used in studies investigating intestinal lipid composition (Mehran *et al.*, 1989; Meddings and Thiesen, 1989; Thomson, *et al.*, 1989; Chu and Walker, 1988; Hübner *et al.*, 1988). Samples (n=3), for each respective diet and IGF-I treatment group, were chosen on the basis of lactase specific activity to determine if differences in brush border membrane lipid composition correlated with high, intermediate, and low levels of lactase activity.

Diet fat has been shown to influence brush border membrane phospholipid fatty acid composition in the small intestine (Rao *et al.*, 1993; Garg *et al.*, 1988; Thomson *et al.*, 1987b,1988b; Clandinin *et al.*, 1985). The changes in fatty acid composition varied between phospholipid classes in this study. It was hypothesized that feeding a diet containing AA would result in increased levels of AA in the brush border membrane. The percentage of C20:4(6) in both phosphatidylcholine and phosphatidylethanolamine did not increase in response to increased dietary intakes of AA by the dam. High levels of AA in the maternal diet have been shown to increase AA content in the gastric contents of suckling rat pups (Perin *et al.*, 1997).

Addition of 1% AA may be capable of changing the composition of breast milk, but this change is small, resulting in an AA level of only 1.5% of total fatty acids (Perin *et al.*, 1997). Previous studies have shown that over time this level of AA in the diet is sufficient to change AA levels in brain and retinal membranes (Clandinin and Van Aerde, 1992). Perhaps these membranes are more readily changeable than those of the small intestine. Trends were observed in regards to higher brush border membrane C20:4(6) levels and dietary AA levels. Thus, higher dietary AA levels may be required to induce significant changes in small intestine brush border membrane C20:4(6) levels.

The hypothesis that the addition of DHA to the dams diet would increase brush border membrane C22:6(3) levels was observed. Phosphatidylethanolamine C22:6(3) levels were significantly higher in the MCT+DHA diet group compared to the diet providing an ω6/ω3 ratio of 4:1. DHA was absent from this diet and low levels of C22:6(3) in membrane are reflected by this absence. Animals and humans possess a limited ability to convert C18:3(3) to C22:6(3) (Layne *et al.*, 1992). The C22:6(3) level for the ω6/ω3 ratio of 4:1 diet group was not different from levels observed in the DHA+AA and MCT+DHA+AA diet treatment groups.

Membranes of suckling pups are very fluid in nature (Brasitus and Dudeja, 1988). As the rat pup matures the membrane becomes less fluid. During the third postnatal week, changes in membrane composition occur, which influence membrane fluidity. An increase in the ratio of saturated to *cis*-unsaturated fatty acids occurs. This steep increase is caused by a sharp decline in the membrane

level of C18:1(9) (Hübner *et al.*, 1988). This may possibly explain the low level of phosphatidylcholine C18:1(7+9) in pups from dams fed the MCT+DHA+AA diet.

Higher levels of membrane C20:4(6) and C22:6(3) were expected in response to dietary MCT incorporation. A previous study by Wang and colleagues (1996) demonstrated significantly higher membrane levels of C20:4(6) and C22:6(3) in weanling rats fed a diet high in MCT. These observations were not consistently observed in phospholipid fractions examined in the present study. Intake of MCT may not affect MCT milk levels. Thus feeding the dam, rather than the pup, a high level of MCT may have resulted in increases in dam C20:4(6) and C22:6(3) intestinal membrane levels, but may not have influenced the levels in the suckling pups.

Effect of IGF-I on Fatty Acid Composition

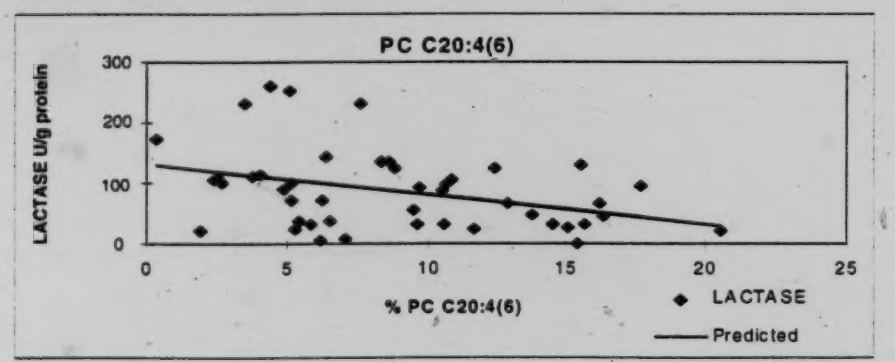
The present study appears to be the first to include a diet variable in combination with IGF-I treatment in suckling rats. Ileal brush border membrane levels of phosphatidylcholine C20:4(6) and phosphatidylethanolamine C22:6(3) were higher in control pups compared to IGF-I treated pups. IGF-I has been shown to enhance absorptive functions and alter structural composition in compromised rat small intestine (Zhang *et al.*, 1995). Thus, it is possible that a relationship between these functional and structural changes may have been influenced by a change in membrane fatty acid composition. Further investigation into this theory is warranted.

Relationship between Lactase Activity and Fatty Acid Levels

Recent studies examining the effects of diet fat on disaccharidase activity levels have found correlations between individual fatty acids and sucrase activity (Dudley *et al.*, 1996; Wang *et al.*, 1996). Dudley and colleagues (1996) observed changes in both sucrase and lactase activity with diet fat manipulation in piglets. To date, changes in lactase activity have not been observed in the rat model (Dudley *et al.*, 1996; Wang *et al.*, 1996). Stenson and colleagues (1989), examined the effects of dietary C20:5(3) and C22:6(3) supplementation, in diets containing low levels of C18:2(6) and devoid of C20:4(6), on sucrase and lactase activities in adult rats. Sucrase and lactase activities were not affected by the addition of C20:5(3) or C22:6(3) (Stenson *et al.*, 1989). The present study demonstrates that the levels of membrane phosphatidylcholine and phosphatidylethanolamine C20:4(6) and phosphatidylcholine C22:6(3) correlate with lactase activity. Lactase activity levels were found to decrease with an increasing level of membrane C20:4(6) **(Figures 5.1 and 5.2)**. The linear regression correlation plot for C22:6(3) demonstrated an increase in lactase levels as C22:6(3) levels rose **(Figure 5.3)**. Previous studies have focused on fat induced disaccharidase changes in adult rats and have not observed changes in lactase activity (Dudley *et al.*, 1996; Wang *et al.*, 1996; Stenson, *et al.*, 1989). Thus, it is possible that the trends observed in this study are only attainable in pre-weaned animals. From these trends, it can be speculated that high levels of membrane C20:4(6), possibly resulting from high levels of ω6 without addition of ω3 fatty acids, may result in decreased levels of

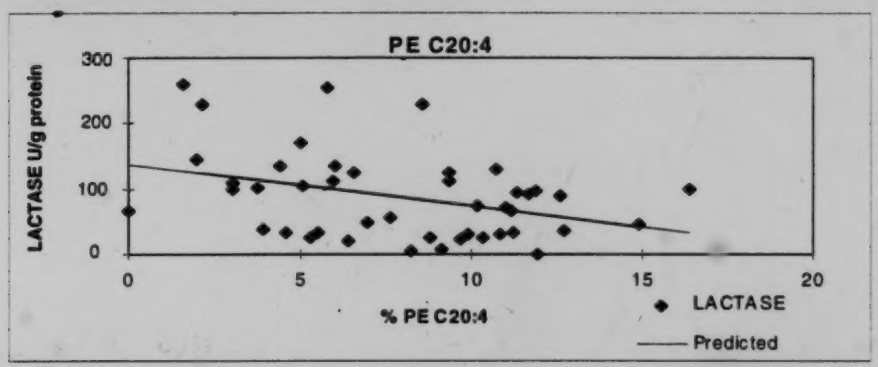
lactase activity prior to weaning. A significant decline in lactase activity prior to the intake of solid food would not be beneficial to the suckling animal. A balance between the two fatty acids, particularly in infant formula where the child is exposed directly to the fatty acids, would be important in regards to maintaining sufficient lactase activity during this period.

Figure 5.1
Relationship Between Lactase Activity and Phosphatidylcholine C20:4(6) Using Data From All Four Diets



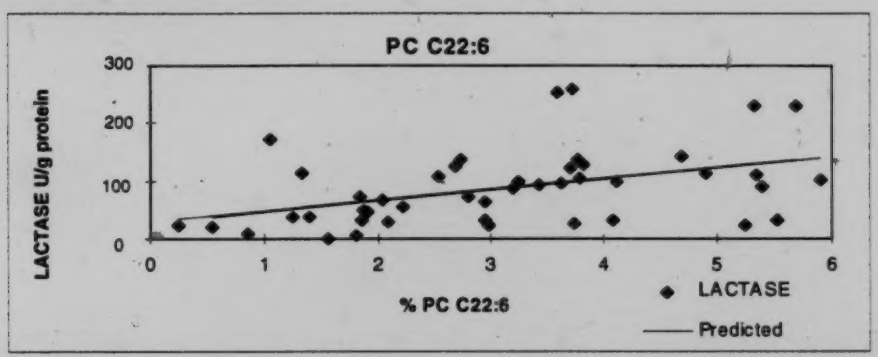
Linear Regression Analysis performed on data from four diet treatments n=45. Lactase activity and phosphatidylcholine C20:4(6) are correlated (r=-0.37, P<0.012). When outlyers were removed, correlation became insignificant.

Figure 5.2
Relationship Between Lactase Activity and Phosphatidylethanolamine C20:4(6) Using Data From All Four Diets



Linear Regression Analysis performed on data from four diet treatments n=44. Lactase activity and phosphatidylethanolamine C20:4(6) are correlated (r=-0.37, P<0.014). When outlyers were removed, correlation became insignificant.

Figure 5.3
Relationship Between Lactase Activity and Phosphatidylcholine C22:6(3) Using Data From All Four Diets



Linear Regression Analysis performed on data from four diet treatments n=45. Lactase activity and phosphatidylcholine C22:6(3) are correlated (r=-0.44, P<0.003). Correlation remained significant with the removal of outlyers (r=0.35, P<0.03).

SUMMARY

The objectives of this study were to examine 1) a potential effect of IGF-I on intestinal disaccharidase activity and 2) the effect of dietary fat manipulation on the fatty acid composition of small intestine brush border membrane phospholipids. Sucrase activity was present in the small intestine, but was not influenced by oral IGF-I treatment. Lactase activity was also unaffected by IGF-I treatment. A relationship was not observed between lactase and sucrase activity. Correlations were observed between lactase activity and membrane levels of C20:4(6) and C22:6(3). These observations were not related to the addition of DHA and AA to the lactating dams diet (lactase expressed as U/g protein). Brush border membrane phospholipid fatty acid composition was altered by maternal diet fat manipulation. The majority of fatty acid changes observed did not conform to the hypotheses stated.

From the results observed, conclusive evidence to suggest a beneficial effect of IGF-I treatment in combination with changes in diet fat intake on small intestine development can not be obtained. Further work into this field of study is necessary. Previous studies investigating the effect of IGF-I on the small intestine have not included diet in their experimental model. A continuation of this study, with extended IGF-I treatment periods, may offer further insight into the effects of IGF-I and diet treatment on disaccharidase activity and membrane fatty acid composition.

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